Dose-dependent and strain-dependent anti-obesity effects of *Lactobacillus sakei* in a diet induced obese murine model

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**Background.** Overweight and abdominal obesity, in addition to medical conditions such as high blood pressure, high blood sugar and triglyceride levels, are typical risk factors associated with metabolic syndrome. Yet, considering the complexity of factors and underlying mechanisms leading to these inflammatory conditions, a deeper understanding of this area is still lacking. Some probiotics have a reputation of a relatively-long history of safe use, and an increasing number of studies are confirming benefits including anti-obesity effects when administered in adequate amounts. Recent reports demonstrate that probiotic functions may widely differ with reference to either intra-species or inter-species related data. Such differences do not necessarily reflect or explain strain-specific functions of a probiotic, and thus require further assessment at the intra-species level. Various anti-obesity clinical trials with probiotics have shown discrepant results and require additional consolidated studies in order to clarify the correct dose of application for reliable and constant efficacy over a long period.

**Methods.** Three different strains of *Lactobacillus sakei* were administered in a high fat diet induced obese murine model using three different doses, \(1 \times 10^{10}\), \(1 \times 10^9\) and \(1 \times 10^8\) CFUs, respectively, per day. Changes in body and organ weight were monitored, and serum chemistry analysis was performed for monitoring obesity associated biomarkers.

**Results.** Only one strain of *L. sakei* (CJLS03) induced a dose-dependent anti-obesity effect, while no correlation with either dose or body or adipose tissue weight loss could be detected for the other two *L. sakei* strains (L338 and L446). The body weight reduction primarily correlated with adipose tissue and obesity-associated serum biomarkers such as triglycerides and aspartate transaminase.

**Discussion.** This study shows intraspecies diversity of *L. sakei* and suggests that anti-obesity effects of probiotics may vary in a strain- and dose-specific manner.
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ABSTRACT

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INTRODUCTION

Overweight and obesity result from abnormal adipose deposition and function and are considered as major pathophysiological symptoms of metabolic syndrome (Olufadi & Byrne, 2008). Originating from insulin resistance, metabolic syndrome may be reflected by several clinical manifestations such as atherosclerosis, hyperglycemia, dyslipidemia, hypertension, reduced high-density lipoprotein cholesterol and type 2 diabetes mellitus (Furukawa et al., 2017). Based on typical pathological symptoms, broadly defined as excessive fat mass in the body (specifically the abdomen), the prevalence of obesity has rapidly increased during the last two decades (Kobyliak et al., 2017). Also referred to as ‘obesity pathogenesis’, obesity is considered as a disorder of the energy homeostasis system rather than the result of passive weight accumulation (Schwartz et al., 2017). In spite of the recent intensive research input, a deeper understanding of pathogenesis and the underlying mechanisms of obesity are still lacking, while, in fact, the causality of obesity has been explained from different viewpoints and disciplines of science such as genetics, endocrinology and psychology (Schwartz et al., 2017).

Following up on classical approaches, recent studies show that the microbiota can play a key role in host obesity and metabolic syndrome (Gérard, 2016). Thereby, new clinical diagnostic perspectives were opened on the influence of the gut microbiota on the status of metabolic disorders. This potential has been highlighted in a review by Boulange et al. (2016), at the same time underlining the complex etiology of these disorders. The current understanding of the mechanisms linking the gut microbiota with metabolic syndrome still appears to be “vague” (Chattophadyay & Mathili, 2018). Indeed, numerous studies have reported on qualitative and quantitative discrepancies in the microbiota of the gastrointestinal tract (GIT) when comparing healthy subjects with people suffering from metabolic diseases (Turnbaugh et al., 2006; Turnbaugh et al., 2008; Ley et al., 2005; Cani & Delzenne, 2009; Armougom et al., 2009).

The International Scientific Association for Probiotics and Prebiotics, after a grammatic correction, has condoned the FAO/WHO consensus definition of probiotics as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Hill et al., 2014). There is general agreement that probiotics support the balance of the
host gut microbiota, and scientific evidence is steadily accumulating regarding the positive
impact of probiotics on human health such as improvement of immune disorders, inflammatory
bowel disease, type 2 diabetes and atherosclerosis (Amar et al., 2011; Kim et al., 2016; Ritze et
al., 2014; Schroeder et al., 2018; Vemuri, Gundamaraju & Eri, 2017). In spite of increasing
evidences of beneficial effects, information is still sparse on the way in which gut microbiota
communicates with distant sites in the host, and also on the mechanisms underlying their
influence on host physiology with regard to (e.g.) the respiratory system, the skin, brain, heart
and host metabolism (Reid et al., 2017). The best recognized mechanisms among the studied
probiotics appear to be related to colonization resistance, acid and short-chain fatty acid (SCFA)
production, regulation of intestinal transit, normalization of perturbed microbiota, increasing
turnover of enterocytes, and competitive exclusion of pathogens (Hill et al., 2014). Using a high-
calorie induced obesity BALB/c mouse model a single strain of Lactobacillus casei IMV B-7280,
and a combination of Bifidobacterium animalis VKL, B. animalis VKB and L. casei IMV B-7280
were shown to be effective in reducing weight gain and cholesterol levels, in the restoration of
liver morphology and in modulating the gut microbiome in a beneficial manner (Bubnov et al.,
2017). However, key issues such as strain-specificity and characterization of dose-dependent
effects still remain to be solved. For this purpose, the further development of both in vitro and
in vivo models appears to be strongly justified. Evidence-based recommendations for probiotics
presently suggest a dose of 10^9 CFU/day or higher (WGO, 2017). A former study involving
volunteers demonstrated a dose of 10^{11} CFU/day (of probiotic strains Bifidobacterium animalis
subsp. lactis BB-12 and Lactobacillus paracasei subsp. paracasei CRL-341) to be effective
(Larsen et al., 2006). For the clinical success of anti-obesity treatment, selection of an optimal
dose and an optimal administration time frame of probiotics are considered to be essential for
inducing beneficial changes, both in gut microbiome diversity and in the metabolism of obese
humans (Bubnov et al., 2017).

Various modes of probiotic action were elucidated by using in vitro studies (including
development of dedicated in vitro models) while efficacy was investigated by both in vivo
(preclinical) studies (Park et al., 2016; Wang et al., 2015) and clinical trials (Kadooka et al.,
2010; Woodard et al., 2009). These therapeutic benefits were all related to anti-obesity effects
of probiotics (Kadooka et al., 2010; Park et al., 2016; Wang et al., 2015; Woodard et al., 2009).

Yet, the anti-obesity efficacy of probiotics has not been fully elucidated in spite of various clinical trials, and scientific evidence for a “minimal dose effect level” remains relatively sparse (Tanentsapf, Heitmann & Adegboye, 2011; Raoult, 2009; Mekkes et al., 2013). The concept of a minimal effective dose is complicated due to the large (and diverse) number of microbial and host-related factors (Salminen et al., 1998), and will also depend on the kind of key criteria and the “end-points” selected. The dose of intolerance is generally considered to be high, thus, allowing a relatively broad “therapeutic window” (Collins, Thornton & Sullivan, 1998), it may be difficult to find a suitably low effective dose above the minimal level. Yet, precisely defining an effective dose has remained an arbitrary issue, and thus the pragmatic suggestion by an FAO/WHO Working Group (FAO/WHO, 2002) that “the suggested serving size must deliver the effective dose of probiotics related to the health claim”. Convincingly delivering this kind of evidence has remained difficult until this day, in particular for commercial distribution of (food or pharmaceutical) strains claimed to be probiotics. In an early report Perdigón, Alvarez & de Ruiz Holgado (1991) suggested a dose related impact of Lactobacillus casei on the secretory immune response and protective capacity in intestinal infections. A placebo-controlled study designed to evaluate the therapeutic value of four different non-antibiotic preparations (including Saccharomyces boulardii, and heat-killed microbial strains) indicated a non-significant dose dependency for either prophylaxis or treatment of traveller’s diarrhoea (Kollaritsch et al., 1989; Kollaritsch et al., 1993). Yet, substantial evidence supports the principle of dose-dependency of probiotics to modulate systemic and mucosal immune function, improve intestinal barrier function, alter gut microbiota, and exert metabolic effects on the host, also in a strain-dependent manner (Alemka et al., 2010; Madsen, 2012). Everard et al. (2011) reported a dose-dependent immunomodulation of human dendritic cells by the probiotic Lactobacillus rhamnosus Lcr35, leading, at high doses, to the semi-maturation of the cells and to a strong pro-inflammatory effect. Against this background, the present study was designed with the challenge of involving a hitherto rarely reported species (Lactobacillus sakei) and its potential for alleviation of obesity [in a diet-induced obese (DIO) mouse model]]. In addition, there was
the prospect of gaining additional insights in intra-species (strain-specific) functional diversity by using established biomarkers.

In this study we administered three different ten-fold dose levels of three different *L. sakei* strains separately to a DIO C57BL/6 murine model and monitored body weight during the full experimental period. Organ weights and serum biomarkers were monitored to elucidate the dose-dependent anti-obesity effect of three different *L. sakei* strains.

**MATERIALS AND METHODS**

**Animal studies**

The animal study was approved by the Ethical Committee of KPC Ltd. in Korea (P150067), in full compliance with ethical standards as specified by Korean law. Five week-old, specific pathogen free (SPF) male C57BL/6 mice were supplied from Orient Bio, Korea. Either a high-fat diet (HFD) (Research Diets D12492) (60 % kcal fat), or low-fat diet (LFD) (Purina Laboratory Rodent Diet 38057) (12 % kcal fat) (negative control) and autoclaved tap water were provided *ad libitum*, while the animals were housed at 23 °C, 55 ± 10 % humidity, in a 12 h light/dark cycle. At the age of 5 weeks mice were fed with either a low-fat control diet containing 12 % kcal of total energy from fat [12.41 % kcal fat, 24.52 % protein, 63.07 % kcal carbohydrate (Purina Laboratory Rodent Diet 38057; Purina Korea Inc., Seoul, Korea)], or a HFD with 60% kcal fat [(90 % of the fat from lard, 10 % from soybean oil), 20 % kcal protein, 20 % kcal carbohydrate (D12492, Research Diets Inc., New Brunswick, NU, USA)] for six weeks. For this study, a HFD of 60 % kcal fat was chosen, as this is one of the most commonly used diets to induce obesity and ectopic lipid storage in *in vivo* studies. Detailed analytical information on the diet composition is given in Table S1 (see also Table 1). The NIH guidelines were followed by providing sufficient cage surface area based on the weight of the mice. In total 120 mice were separated into 12 different groups (5 animals per cage and two cages per group) with each group receiving a different treatment. Study design is given in Table 2 and information on the diets in Table 1.

// Insert Table 1 //
The experiment comprised one week of adaptation followed by six weeks of obesity induction using a HFD while the LFD group was maintained on LFD feeding. A total number of 110 mice received the test substances, with exception of those with the upper and lower body weights after the six-week period of obesity induction. All treatments were by oral gavage and were performed twice a day, at the same daytime (10:00 and 17:00), for seven weeks. Each group was treated with either the microbial culture suspended in PBS, orlistat suspended in PBS, as chemical control, or only PBS as negative control. Orlistat was provided as Xenical (with 120 mg/g of orlistat as active pharmaceutical ingredient, and microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, povidone, and talc as inactive ingredients). The contents of the Xenical capsules were added to PBS, as explained in Table 1. As orlistat is insoluble in water, it was suspended by vortexing and sonication and then orally administered to the animals. For oral administration each microbial strain was washed twice with PBS and the supernatant discarded after centrifugation. The microbial pellet was resuspended in PBS to suit the dose for administration. On the last day of the experiment, the mice were sacrificed by dislocation of the cervical vertebra. The organs, i.e., liver, femoral muscle, brown adipose tissue, epididymal adipose tissue, subcutaneous adipose tissue and mesenteric adipose tissue were collected, weighed, and stored at -80 °C. Each perfused liver was embedded in paraffin and sectioned (4 μm) on a microtome. Hematoxylin and eosin (H&E) staining was performed on each high dose L. sakei group and assessed by light microscopy (Olympus MVX10 microscope, equipped with a DC71 camera, Center Valley, PA. Olympus, Japan).

Serum triglycerides (TG), glucose (GLU), total cholesterol (TC), high density lipoprotein (HDL), low-density lipoprotein (LDL), and aspartate transaminase [AST; a marker of liver toxic injuries of hepatocytes (Aulbach and Amuzie. 2017)], were measured using an automated biochemical analyser BS-200 (Mindray, China) in Pohang Technopark, Pohang (South Korea).

**Microorganisms**
Lactobacillus sakei strain CJLS03 was isolated from kimchi, while L. sakei strains CJB38 and CJB46 originated from human fecal samples. These strains were selected among 9 different strains (comprising 4 Lactobacillus brevis, 3 L. sakei, 1 Lactobacillus plantarum and 1 Bifidobacterium longum) on the basis of the lowest weight gain in a preliminary study using a DIO mouse model (data shown in Fig. S1).

The 3 L. sakei strains were grown daily in MRS broth (Difco Laboratories INC., Franklin Lakes, NJ, USA) for feeding during the seven-week period of intervention. Strains were grown for 8 h to reach their late log phase and were collected by centrifugation (3546 g, 5 min, 5 °C) (Centrifuge: Hanil Science Industry, Korea) and washed two times with PBS. Each strain was prepared in an approximate number of $1 \times 10^{10}$ CFU/ml using a mathematical equation derived from a pre-optimised standard curve (Fig. S2) using optical density by SPECTROstar Nano (BMG Labtech, Durham, USA). A stock suspension of $1 \times 10^{10}$ CFU/mL (high-dose, H) was prepared of each strain, then diluted ten-fold to $1 \times 10^9$ (medium-dose, M) and $1 \times 10^8$ CFU/mL (low-dose, L), respectively, and finally suspended in 300 µl of PBS to be administered to each mouse by oral gavage.

Experimental determinants were statistically calculated using ANOVA and Dunnett’s multiple comparison test to distinguish the level of significance based on probability of 0.05 (*), 0.01 (**) and 0.001 (***)

RESULTS

HFD feeding resulted in a strong increase in body mass as compared to those animals receiving LFD administration (Fig. 1A) over the 48-day feeding period. Moreover, elevated levels of serum biomarkers such as TG, TC, GLU, LDL and AST were detected in the HFD group (Fig. 2), concomitantly with quantitative increases in epididymal, mesenteric and subcutaneous adipose tissues (Fig. 3). Orlistat therapy did not cause any mentionable side-effects in the treated animals. No animals in any of the groups died during the study period.

Three different doses ($10^8$-$10^{10}$) of the three L. sakei strains (CJB38, CJB46 and CJLS03) were orally administered to high fat DIO C57BL/6 mice for 7 weeks, and body weight and food consumption were measured daily. During the test period, 3 strains were found to exhibit
reduced weight gain compared to the HFD group (Fig. 1 B, C, D), with strain CJLS03 showing, dose-dependently, the strongest effect of the 3 strains. LFD, Orlistat, the full CJB46 group, and medium and high dose of the CJLS03 groups showed significantly lower weight increase compared to the HFD group (Fig. 1 E; Fig. S3). The weight loss of CJB38 or CJB46 was not dependent of the dose while only strain CJLS03 showed a dose-dependent weight reduction effect, and with the highest efficacy of all groups for CJLS03 H (Fig. 1 E). The onset time of weight loss showed significance compared to the HFD at days 4, 21, 21 and 7 for the Orlistat, CJB38, CJB46 and CJLS03 groups, respectively (Table S2). The daily dietary intake was significantly higher in the LFD, Orlistat and CJLS03 M groups compared to the HFD group (Fig. 1 F).

Serum biochemical analysis showed an overall increase in the lipid profile (TC, TG, HDL, LDL), liver (AST) and the GLU level of the HFD group compared to the LFD group, demonstrating that a HFD intake may impact various biomarkers associated with pathophysiological symptoms of obesity (Fig. 2). Compared to the HFD group, the serum TG level decreased in all test groups (Fig. 2 A) while the LDL level was significantly reduced in all test groups except CJB46 H (Fig. 2 E). Significant reduction of TC was only detected in LFD, Orlistat and in the groups treated with higher doses (M and H) of *L. sakei* CJB38 H, CJB46 M, CJB46 H, CJLS03 M and CJLS03 H (Fig 2 C). In particular, the CJLS03 group, shown to be superior regarding weight gain inhibition, appears to be effective in a dose-dependent manner (Fig. 2 A, B, C). HDL levels were not significantly different from the HFD group in all the test groups, however, all *L. sakei* treated groups except CJB46 L, CJLS03 M and CJLS03 H showed significant increase when the ratio of HDL to total cholesterol level was calculated; this is reflected in Fig. 2D. Serum AST values (indicating liver function) were found to be approximately 1.7 times higher for the HFD compared to the LFD group (Fig. 2 F), while the Orlistat group showed no significant change in AST level compared to the HFD group. All nine groups receiving the *L. sakei* strains showed a trend towards reduced AST levels but with only the high dose of CJLS03 (CJLS03 H) differing significantly when compared to the HFD group (Fig. 2 F). CJLS03 showed the highest overall effectivity and a dose-dependent anti-obesity function; at the same time, it induced a dose-dependent improvement of serum obesity-associated biomarkers and liver function. Liver H&E staining optically
demonstrated normal histology in LFD mice with minor lipid accumulation. Comparing the visual differences, the HFD-fed mice showed extensive fat accumulation and moderate vacuolations around the portal triad. In the groups treated with the higher dose of \textit{L. sakei} CJB38 H, CJB46 H and CJLS03 H inhibition of lipid accumulation was visually evident and was comparable to that of the LFD group (Fig. S4).

Compared to HFD the LFD group showed significantly lower weights of epididymal, mesenteric, subcutaneous and brown adipose tissues while insignificant organ weight differences were measured in liver and femoral muscles (Fig 3). Every dose of all three strains of \textit{L. sakei} and the orlistat treatment resulted in significantly lower subcutaneous adipose tissue weight while only CJLS03 H showed significant reduction of visceral adipose tissue including epididymal and mesenteric adipose tissue, when compared to the HFD group (Fig. 3 A, B, C). CJLS03 M treatment significantly reduced epididymal adipose tissue weight when compared to the HFD group (Fig 3 a). These results suggest that the three different \textit{L. sakei} strains inhibited the accumulation of subcutaneous adipose tissue but that the CJLS03 group responded by dose-dependent reduction of visceral adipose tissues including the epididymal and mesenteric adipose tissues (Fig. 3 A, B). Orlistat and \textit{L. sakei} treatment did not result in significant weight differences regarding brown adipose tissue, liver and femoral muscle (Fig. 3 D, E, F).

DISCUSSION

The impact of a HFD on various biomarkers associated with pathophysiological symptoms of obesity is well established and supported in current literature (\textit{Chandler et al., 2017}; \textit{Lee, 2013}; \textit{Ludwig et al., 2018}; \textit{Siri-Tarino et al., 2010}). The body mass increase resulting from HFD feeding (as compared to a LFD) in this study (Fig. 1) was also accompanied by significant increases in serum biomarkers such as TG, TC, GLU, LDL and AST (Fig. 2) and also increases in epididymal, mesenteric and subcutaneous adipose tissues (Fig. 3). Definition of an ideal HFD and its exact composition is generally considered difficult (Buettner et al., 2007). Essential is, however, the
standardization of the specific laboratory and feeding conditions for the purpose of metabolic studies. In our studies, we have used exactly defined and commercially available high-fat and low-fat diets (HFD and LFD). The selected murine model (male C57BL/6 mice) is widely preferred as in vivo model for obesity and metabolic studies (Khan et al., 2014) and related investigations (Neuhofer et al., 2014).

The anti-obesity influence of administered probiotics is a heavily debated issue, yet, an indisputable fact is that the host gut microbiota is exercising a leverage over energy efficiency and adipose tissue accumulation (Kobyliak et al., 2017; Greiner and Bäckhed, 2011; Delzenne et al., 2011). At the same time, probiotics have been reported to impact the host microbiota in a positive way (Hemarajata and Versalovic, 2013) and to beneficially influence gut homeostasis and reduce the symptoms of gastrointestinal diseases (Bron et al., 2017). The beneficial effect of probiotics on the levels of alanine aminotransferase, aspartate transaminase (AST), TC, HDL, tumor necrosis factor (TNF)-\(\alpha\) and also on insulin resistance [assessed in a homeostasis model (HOMA-IR)] have been reported earlier (Ma et al., 2013). In a study using C57BL/6J mice Lactobacillus rhamnosus GG (LGG) showed a protective effect against nonalcoholic fatty liver disease (NAFLD) induced by a high-fructose diet (Ritze et al., 2014). This potential is supported by meta-analysis of data from randomized controlled trials in patients with NAFLD, showing probiotic therapy to result in a significant decrease of NAFLD (Ma et al., 2013; Al-muzafar and Amin, 2017). Moreover, probiotic therapy has been shown to be typically associated with a reduction in liver aminotransferase levels (Aller et al., 2011; Buss et al., 2014; Shavakhi et al., 2013). The significant reduction of liver AST levels by L. sakei CJLS03 H in our study suggests its possible therapeutic potential for alleviation of NAFLD. The potential advantages of probiotics as complementary treatment for metabolic disorders and as therapy for NAFLD are increasingly recognized (Le Barz et al., 2015; Ma et al., 2017). Moreover, the modulatory effect of probiotics on the gut microbiota suggests their potential as a “promising and innovative add-on therapeutic tool” for the treatment of NAFLD (Paolella et al., 2014). In our study, inhibition of hepatic lipid accumulation in HFD animals was revealed by Liver H&E staining and was particularly obvious for the groups treated with orlistat and CJLS03 H which also compared well with the normal histological features of the LFD group (Fig. S4).
The function of orlistat in assisting weight loss is well established and has been supported by Cochrane meta-analysis of various randomized controlled trials (Drew, Diuxon & Dixon, 2007). Obesity control may be by several mechanisms, one of which being that orlistat prevents fat hydrolysis by acting as a gastric and pancreatic lipase inhibitor (Heck, Yanovski & Calis, 2012; Yanovski & Yanovski, 2014). It has been successfully used as anti-obesity control in animal experiments involving high fat DIO rats (Karimi et al., 2015) and DIO C57BL/6 mice (Chung et al., 2016). The latter studies also included clinical trials, and the authors (Chung et al., 2016) claimed orlistat to be the most popular anti-obesity pharmaceutical drug, both in animal (DIO C57BL/6 mice) experiments and clinical trials. The DIO C57BL/6 mouse is now widely accepted as an in vivo model of choice. It has been reported to closely reflect human metabolic disorders such as obesity, hyperinsulinemia, hyperglycemia and hypertension (Collins et al., 2004). Especially the metabolic abnormalities of DIO C57BL/6 after HFD feeding are considered reported to closely resemble those of human obesity development patterns (Speakman et al., 2007), and also regarding properties such as adipocyte hyperplasia, fat deposition in the mesentery and increased fat mass (Inui., 2003).

Probiotic administration increasingly enjoys consideration as a promising approach for beneficially modulating the host microbiota (Jia, Zhao & Nicholson, 2008; Steer et al., 2000). Numerous reports confirmed the beneficial effects of specific probiotic strains against diarrhoea and inflammatory bowel diseases (Ahmadi, Alizadeh-Navaei & Rezai 2015; Gionchetti et al., 2000; Ouwehand, Salminen & Isolauri, 2002). Recently, anti-obesity effects of probiotics were also reported and confirmed in clinical trials (Kadooka et al., 2010; Woodard et al., 2009; Minami et al., 2015; 2018; Borgeraas et al., 2017) and animal models (Kim et al., 2016; Alard et al., 2016; Wang et al., 2015; Ji et al., 2012). Kadooka et al. (2010) investigated the anti-obesity effect of the probiotic Lactobacillus gasseri SBT2055 by conducting a double-blind, randomised, placebo-controlled intervention trial with 87 overweight and obese subjects for 12 weeks. The data confirmed that the abdominal visceral and subcutaneous fat area, weight, BMI, as well as waist and hip measures were significantly reduced in the group consuming the probiotic. In another study (Woodard et al., 2009) 44 morbid obese patients were operated for weight loss by surgery (gastric bypass surgery) and were randomly divided in a probiotic administered
group and a control group. A significantly higher weight loss was recorded in the group receiving the probiotic (described as “Puritan’s Pride®”, containing a mixture of 2.4 billion live cells of *Lactobacillus* spp.). Park et al. (2013) reported a significant weight reduction of a C57BL/6 mice model after *Lactobacillus curvatus* HY7601 and *L. plantarum* KY1032 consumption, however, faecal microbiota modulation of major groups such as *Firmicutes* and *Bacteroidetes* was not monitored.

One of the major hurdles for an accurate clinical trial is to understand the effective dose of a probiotic at a strain-specific level. Selecting the correct dose of a probiotic for a specific purpose such as the alleviation of diarrhoea was suggested in various studies, yet, there is a general lack of scientific proof of a concept to define the functional dose of a probiotic (Kollaritsch et al., 1993; Kollaritsch et al., 1989; Islam, 2016). Chen et al. (2015) used a range of 5 different tenfold doses of *Lactobacillus acidophilus* in a colitis-induced animal model and reported $10^6$ CFU/10 g of the animal weight as the most effective application level for modulating the bacterial profile in the distal colon. In our study we have monitored dose-related effects of three different strains of *L. sakei* and found only one strain, CJLS03, to show a dose-dependent anti-obesity effect while the anti-obesity impact of the other two strains was lower and dose-independent (Fig. S3). At dose levels from $1 \times 10^8$ to $1 \times 10^{10}$ CFU/mL administration of strain CJLS03 resulted in a dose-related (progressive) reduction in the levels of TC, TG, AST, mesenteric adipose tissue and epididymal adipose tissue (Fig. S3). Adipose tissues were reduced relative to weight gain, and TG and TC showed the most significant reduction in the *L. sakei* treated groups compared to the HFD control group. Another *L. sakei* strain (OK67) isolated from kimchi was reported to ameliorate HFD-induced blood glucose intolerance and obesity in mice; mechanisms for this effect have been suggested to be by inhibition of gut microbial lipopolysaccharide production and the inducing of colon tight junction protein expression (Lim et al., 2016).

Our study has confirmed the relevance of a strain-specific approach when selecting functional strains suitable for (costly and time-consuming) clinical studies. The importance of this issue has been emphasized in recent papers with regard to pre-clinical physiological studies on putative probiotic strains of lactic acid bacteria and *Bifidobacterium*. These studies involved...
features such as adhesion potential, antibiotic resistance and survival under simulated conditions of the upper GIT, in addition to the modulation of the gut microbiome (Bubnov et al., 2018).

CONCLUSIONS

This in vivo investigation showed that beneficial effects of putative probiotics are both strain-specific and dose-related. For only one (CJLS03) out of three L. sakei strains an anti-obesity effect could be detected, which, at the same time, was found to be dose-dependent. The highest of three doses (1 x 10^{10} CFU/day) of CJLS03 gave the most favourable (significant) biomarker-related effects with regard to cholesterol and triglyceride reduction, when compared to the HFD control.

Supplemental Information

Supplemental information for this article can be found online at.....

REFERENCES

Ahmadi E, Alizadeh-Navaei R, Rezai MS. 2015. Efficacy of probiotic use in acute rotavirus diarrhea in children: A systematic review and meta-analysis. Caspian Journal of Internal Medicine. 6: 187.

Alard J, Lehrter V, Rhimi M, Mangin I, Peucelle V, Abraham AL, Mariadassou M, Maguin E, Waligora-Dupriet AJ, Pot B, Wolowczuk I, Grangette C. 2016. Beneficial metabolic effects of selected probiotics on diet-induced obesity and insulin resistance in mice are associated with improvement of dysbiotic gut microbiota. Environmental Microbiology 18: 1484-1497 https://doi.org/10.1111/1462-2920.13181.

Alemka A, Clyne M, Shanahan F, Tompkins T, Corcionivoschi N, Bourke B. 2010. Probiotic
colonization of the adherent mucus layer of HT29MTXE12 cells attenuates
*Campylobacter jejuni* virulence properties. *Infection and Immunity* 78: 2812-2822
DOI:10.1128/IAI.01249-09.

Aller R, De Luis DA, Izaola O, Conde R, Gonzalez Sagrado M, Primo D, De La Fuente B,
Gonzalez J. 2011. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty
liver disease patients: A double blind randomized clinical trial. *European Review for
Medical and Pharmacological Sciences* 15: 1090–1095.

Al-muzafar HM, Amin KA. 2017. Probiotic mixture improves fatty liver disease by virtue of its
action on lipid profiles, leptin, and inflammatory biomarkers. BMC Complementary and
Alternative Medicine 17: 43; DOI 10.1186/s12906-016-1540-z.

Amar J, Chabo C, Waget A, Klopp P, Vachoux C, Bermudez-Humaran LG, Smirnova N, Berge M,
Sulpice T, Lahtinen S, Ouwehand A, Langella P, Rautonen N, Sansonetti P, Burcelin R.
2011. Intestinal mucosal adherence and translocation of commensal bacteria at the
early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. *EMBO
Molecular Medicine* 3: 559-572 DOI:10.1002/emmm.201100159.

Armougom F, Henry M, Vialettes B, Raccach D, Raoul D. 2009. Monitoring bacterial community
of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and
Methanogens in anorexic patients. *PLoS One* 4: e7125
DOI:10.1371/journal.pone.0007125.

Aulbach AD, Amuzie CJ. 2017. *Biomarkers in Nonclinical Drug Development*. In: A
Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition),
Chapter 17, pp. 447-471. London: Academic Press (Elsevier).
https://doi.org/10.1016/B978-0-12-803620-4.00017-7.

Borgeraas H, Johnson LK, Skattebu J, Hertel JK, Hjelmesæth J. 2017. Effects of probiotics on
body weight, body mass index, fat mass and fat percentage in subjects with overweight
or obesity: a systematic review and meta-analysis of randomized controlled trials.
*Obesity Reviews* 19: 219–232. doi: 10.1111/obr.12626.
Boulange CL, Neves AL, Chilloux J, Nicholson JK, Dumas M-E. 2018. Impact of the gut microbiota on inflammation, obesity and metabolic disease. *Genome Medicine* 8:42. DOI 10.1186/s13073-016-0303-2.

Bron PA, Kleerebezem M, Brummer R-J, Cani PD, Mercenier A, MacDonald TT, Garcia-Ródenas CL, Wells JM. 2017. Can probiotics modulate human disease by impacting intestinal barrier function? *British Journal of Nutrition* 117: 93–107; doi:10.1017/S0007114516004037.

Bubnov RV, Babenko LV, Lazarenko LM, Mokrozub VV, Demchenko OA, Nechypurenko OV, Spivak MY. 2017. Comparative study of probiotic effects of *Lactobacillus* and Bifidobacteria strains on cholesterol levels, liver morphology and the gut microbiota in obese mice. *EPMA Journal* 8(7): 357-376. DOI 10.1007/s13167-017-0117-3.

Bubnov RV, Babenko LV, Lazarenko LM, Mokrozub VV, Spivak MY. 2018. Specific properties of probiotic strains: relevance and benefits for the host. *EPMA Journal* 9(2):205-223. doi: 10.1007/s13167-018-0132-z.

Buettner R, Schölmerich J, Bollheimer LC. 2007. High-fat Diets: Modeling the Metabolic Disorders of Human Obesity in Rodents. *Obesity* 15(4): 798-808.

Buss C, Valle-Tovo C, Miozzo S, Alves de Mattos A. 2014. Probitoics and synbiotics may improve aminotransferases levels in non-alcoholic fatty liver disease patients. *Annals of Hepatology* 13(5): 482-488.

Cani PD, Delzenne NM. 2009. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. *Current Opinion in Pharmacology* 9: 737-743 https://doi.org/10.1016/j.coph.2009.06.016.

Chandler M, Cunningham S, Lund EM, Khanna C, Naramore R. Patel A, Day MJ. 2017. Obesity and Associated Comorbidities in People and Companion Animals: A One Health Perspective. *Journal of Comparative Pathology* 156: 296-309. http://dx.doi.org/10.1016/j.jcpa.2017.03.006.

Chattophadyay A, Mathili, S. 2018. The journey of gut microbiome – An introduction and its
influence on metabolic disorders. *Frontiers in Biology* **13**: 327-341.

https://doi.org/10.1007/s11515-018-1490-6.

Chen L, Zou Y, Peng J, Lu F, Yin Y, Li F, Yang J. 2015. *Lactobacillus acidophilus* suppresses colitis-associated activation of the IL-23/Th17 axis. *Journal of Immunology Research* Volume 2015, Article ID 909514, 10 pages http://dx.doi.org/10.1155/2015/909514

Chung H-J, Yu JG, Lee I-A, Liu M-J, Shen Y-F, Sharma SP, Jamal MAHM, Yoo J-H, Kim H-J, Hong S-T. 2016. *FEBS Open Bio* **6**: 64-76, doi:10.1002/2211-5463.12024.

Collins JK, Thornton G, Sullivan GO. 1998. Selection of probiotic strains for human applications. *International Dairy Journal* **8**: 487-490 https://doi.org/10.1016/S0958-6946(98)00073-9.

Collins S, Martin TL, Surwit RS, Robidoux J. 2004. Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. *Physiology & Behavior* **81**:243–248. https://doi.org/10.1016/j.physbeh.2004.02.006

Delzenne NM, Neyrinck AM, Bäckhed F, Cani PD. 2011. Targeting gut microbiota in obesity: effects of prebiotics and probiotic. *Nature Reviews in Endocrinology* **7**: 639-646 DOI:10.1038/nrendo.2011.126.

Drew BS, Diuxon AF, Dixon JB. 2007. Obesity management: Update on orlistat. *Vascular Health Risk Management* **3**(6): 817-821.

Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, Possemiers S, Van Holle A, François P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD. 2011. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **60**: 2775-2786 DOI: 10.2337/db11-0227.

Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. 2017. Increased oxidative stress in obesity and its impact on metabolic syndrome. *The Journal of Clinical Investigation* **114**: 1752-1761 DOI:10.1172/JCI200421625.

Gérard P. 2016. Gut microbiota and obesity. *Cellular and Molecular Life Sciences* **73**: 147-162 DOI 10.1007/s00018-015-2061-5.
Gionchetti P, Rizzello F, Venturi A, Campieri M. 2000. Probiotics in infective diarrhoea and inflammatory bowel diseases. Journal of Gastroenterology and Hepatology 15: 489-493.

Greiner T, Bäckhed F. 2011. Effects of the gut microbiota on obesity and glucose homeostasis. Trends in Endocrinology and Metabolism 22: 117-123 DOI:
https://doi.org/10.1016/j.tem.2011.01.002

Heck AM, Yanovski JA, Calis KA. 2012. Orlistat, a New Lipase Inhibitor for the Management of Obesity. Pharmacotherapy 20(3): 270-279.
https://doi.org/10.1592/phco.20.4.270.34882

Hemarajata P, Versalovic J. 2013. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therapeutic Advances in Gastroenterology 6(1): 39-51; DOI: 10.1177/1756283X12459294.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC, Sander ME. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. National Reviews on Gastroenterology and Hepatology 11: 506-514. doi:10.1038/nrgastro.2014.66.

Inui A. 2003. Obesity – a chronic health problem in cloned mice? Trends in Pharmacological Sciences 24: 77–80. https://doi.org/10.1016/S0165-6147(02)00051-2.

Islam SU. 2016. Clinical uses of probiotics. Medicine 95(5) DOI:
10.1097/MD.0000000000002658.

Ji YS, Kim HN, Park HJ, Lee JE, Yeo SY, Yang JS, Park SY, Yoon HS, Cho GS, Franz CM, Bomba A, Shin HK, Holzapfel WH. 2012. Modulation of the murine microbiome with a concomitant anti-obesity effect by Lactobacillus rhamnosus GG and Lactobacillus sakei NR28. Beneficial Microbes 3: 13-22 DOI 10.3920/BM2011.0046.

Jia W, Li H, Zhao L, Nicholson JK. 2008. Gut microbiota: a potential new territory for drug targeting. Nature Reviews in Drug Discovery 7: 123-129 doi:10.1038/nrd2505.

Kadooka YM, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M,
Tsuchida T. 2010. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. European Journal of Clinical Nutrition 64: 636-643 DOI 10.1038/ejcn.2010.19.

Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, Khazaai H, Khodavandi A. 2015. The anti-obesity effects of Lactobacillus casei strain Shirota versus Orlistat on high fat diet-induced obese rats. Food and Nutrition Research 59: 29273, http://dx.doi.org/10.3402/fnr.v59.29273.

Khan M, Patrick AL, Fox-Robichaud AE, The Canadian Critical Care Translational Biology Group. 2014. Development of a Murine Model of Early Sepsis in Diet-Induced Obesity. Biomed Research International. Volume 2014, Article ID 719853, 11 pages. http://dx.doi.org/10.1155/2014/719853.

Kim B, Park K-Y, Ji Y, Park S, Holzapfel W, Hyun C-K. 2016. Protective effects of Lactobacillus rhamnosus GG against dyslipidemia in high-fat diet-induced obese mice. Biochemical and Biophysical Research Communications 473: 530-536. http://dx.doi.org/10.1016/j.bbrc.2016.03.107.

Kobyliak N, Falalyeyeva T, Beregova T, Spivak M. 2017. Probiotics for experimental obesity prevention: focus on strain dependence and viability of composition. Endokrynologia Polska, 68: 659-667 DOI: 10.5603/EP.a2017.0055.

Kollaritsch HH, Holst H, Grobara P, Wiedermann G. 1993. Prevention of traveler's diarrhea with Saccharomyces boulardii. Results of a placebo controlled double-blind study. Fortschritte der Medizin 111: 152-156.

Kollaritsch HH, Kremsner P, Wiedermann G, Scheiner O. 1989. Prevention of traveller’s diarrhea: comparison of different non-antibiotic preparation. Travel Medicine International 6: 9-17.

Larsen CN, Nielsen S, Kaestel P, Brockmann E, Bennedsen M, Christensen HR, Eskesen DC, Jacobsen BL, Michaelsen KF. 2006. Dose-response study of probiotic bacteria Bifidobacterium animalis subsp. lactis BB-12 and Lactobacillus paracasei subsp.
paracasei CRL-341 in healthy young adults. *European Journal of Clinical Nutrition* **60**(11): 1284–1193.

Larsen N, Vogensen FK, Gobel RJ, Michaelsen KF, Forssten SD, Lahtinen SJ, Jakobsen M. 2013. Effect of *Lactobacillus salivarius* Ls-33 on fecal microbiota in obese adolescents. *Clinical Nutrition* **32**: 935-940. https://doi.org/10.1016/j.clnu.2013.02.007.

Le Barz M, Anhé FF, Varin TV, Desjardins Y, Levy E, Roy D, Urdaci MC, Marette A. 2015. Probiotics as Complementary Treatment for Metabolic Disorders. *Diabetes & Metabolism Journal* **39**: 291-303. http://dx.doi.org/10.4093/dmj.2015.39.4.291.

Lee CY. 2013. The Effect of High-Fat Diet-Induced Pathophysiological Changes in the Gut on Obesity: What should be the Ideal Treatment? *Clinical and Translational Gastroenterology* **4**: e39; doi:10.1038/ctg.2013.11.

Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. 2005. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Science of the USA* **102**: 11070-11075. www.pnas.org/cgi_doi_10.1073_pnas.0504978102.

Lim SM, Jeong JJ, Woo, KH, Han MJ, Kim DH. 2016. *Lactobacillus sakei* OK67 ameliorates high-fat diet–induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression. *Nutrition Research* **36**(4): 337-348. https://doi.org/10.1016/j.nutres.2015.12.001.

Ludwig DS, Willett WC, Volek JS, Neuhouser ML. 2018. Dietary fact: From foe to friend? *Science* **362**: 764-770. DOI: 10.1126/science.aau2096.

Ma J, Zhou Q, Li H. 2017. Gut Microbiota and Nonalcoholic Fatty Liver Disease: Insights on Mechanisms and Therapy. *Nutrients* **9**: 1124; doi:10.3390/nu9101124.

Ma Y-Y, Li L, Yu C-H, Shen Z, Chen L-H, Li Y-M. 2013. Effects of probiotics on nonalcoholic fatty liver disease: A meta-analysis. *World Journal of Gastroenterology* **19**(40): 6911-6918. doi:10.3748/wjg.v19.i40.6911.

Madsen KL. 2012. Enhancement of epithelial barrier function by probiotics. *Journal of Epithelial
Mekkes MC Weenen TC, Brummer RJ, Claassen E. 2013. The development of probiotic treatment in obesity: a review. Beneficial Microbes 5: 19-28.

Minami J, Kondo S, Yanagisawa N, Odamak T, Xiao J, Abe F, Nakajima S, Hamamoto Y, Saitoh S, Shimoda T. 2015. Oral administration of Bifidobacterium breve B-3 modifies metabolic functions in adults with obese tendencies in a randomised controlled trial. Journal of Nutritional Science 4: 1-7; doi:10.1017/jns.2015.5.

Minami J, Iwabuchi N, Tanaka M, Yamauchi K, Xiao J, Abe F, Sakane N. 2018. Effects of Bifidobacterium breve B-3 on body fat reductions in pre-obese adults: A randomized, double-blind, placebo-controlled trial. Bioscience of Microbiota, Food and Health 37: 67-75. DOI: 10.12938/bmfh.18-001.

Neuhofer A, Wernly B, Leitner L, Sarai A, Sommer NG, Staffler G, Zeyda M, Stulnig TM. 2014. An accelerated mouse model for atherosclerosis and adipose tissue inflammation. Cardiovascular Diabetology 13: 23. http://www.cardiab.com/content/13/1/23.

Olufadi R, Byrne CD. 2008. Clinical and laboratory diagnosis of the metabolic syndrome. Journal of Clinical Pathology 61: 697-706 http://dx.doi.org/10.1136/jcp.2007.048363.

Ouwehand AC, Salminen S, Isolauri E. 2002. Probiotics: an overview of beneficial effects. Antonie van Leeuwenhoek 82: 279-289.

Paolella G, Mandato C, Pieri L, Poeta M, Di Stasi M, Vajro P. 2014. Gut-liver axis and probiotics: Their role in non-alcoholic fatty liver disease. World Journal of Gastroenterology 20(42): 15518-15531. DOI: http://dx.doi.org/10.3748/wjg.v20.i42.15518.

Park DY, Ahn YT, Park SH, Huh CS, Yoo SR, Yu R, Sung MK, McGregor RA, Choi MS. 2013. Supplementation of Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY1032 in diet-induced obese mice is associated with gut microbial changes and reduction in obesity. PLoS One 8: e59470 doi:10.1371/journal.pone.0059470.
Park S, Ji Y, Park H, Lee K, Park H, Beck BR, Shin H, Holzapfel WH. 2016. Evaluation of functional properties of lactobacilli isolated from Korean white kimchi. *Food Control* **69**: 5-12 DOI: 10.1016/j.foodcont.2016.04.037.

Perdigón G, Alvarez S, de Ruiz Holgado AP. 1991. Immunoadjuvant activity of oral *Lactobacillus casei*: influence of dose on the secretory immune response and protective capacity in intestinal infections. *Journal of Dairy Research* **58**: 485-496.

Raoult D. 2009. "Probiotics and obesity: a link?" *Nature Reviews Microbiology* **7**(9): 616 DOI 10.1038/nrmicro2209.

Reid G, Abrahamsson T, Bailey M, Bindels LB, Bubnov R, Ganguli K, Martoni C, O’Neill C, Savignac HM, Stanton C, Ship N, Surette M, Tuohy K, Van Hemert S. 2017. How do probiotics and prebiotics function at distant sites? *Beneficial Microbes* **8**(4): 521-533. https://doi.org/10.3920/BM2016.0222.

Ritze Y, Bárdos G, Claus A, Ehrmann V, Bergheim I, Schwertz A, Bischoff SC. 2014. *Lactobacillus rhamnosus* GG protects against non-alcoholic fatty liver disease in mice. *PLoS One* **9**: e80169 doi:10.1371/journal.pone.0080169.

Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fondén R, Saxelin M, Collins K, Mogensen G. 1998. Demonstration of safety of probiotics—a review. *International Journal of Food Microbiology* **44**: 93-106.

Schroeder BO, Birchenough GMH, Stahlman M, Arike L, Johansson MEV, Hansson GC, Bäckhed F. 2018. Bifidobacteria or Fiber Protects against Diet-Induced Microbiota-Mediated Colonic Mucus Deterioration. *Cell Host Microbe* **23**: 27-40 e7 https://doi.org/10.1016/j.chom.2017.11.004.

Schwartz MW, Seeley TJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM, Leibel RL. 2017. Obesity pathogenesis: an Endocrine Society scientific statement. *Endocrine Reviews* **38**: 267-296 doi: 10.1210/er.2017-00111.

Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G. 2013. Effect of a Probiotic and Metformin on Liver Aminotransferases in Non-alcoholic Steatohepatitis: A
Double Blind Randomized Clinical Trial. *International Journal of Preventive Medicine* 4: 531–537.

**Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. 2010.** Saturated fat, carbohydrate, and cardiovascular disease. *American Journal of Clinical Nutrition* 91: 502-509.

**Speakman J, Hambly C, Mitchell S, Krol E. 2007.** Animal models of obesity. *Obesity Reviews* 8 (Suppl. 1): 55–61. DOI: [10.1111/j.1467-789X.2007.00319.x](http://dx.doi.org/10.1111/j.1467-789X.2007.00319.x).

**Steer TH, Carpenter H, Tuohy K, Gibson GR. 2000.** Perspectives on the role of the human gut microbiota and its modulation by pro- and prebiotics. *Nutrition Research Reviews* 13: 229-254 DOI 10.1079/095442200108729089.

**Tanentsapf I, Heitmann BL, Adegboye ARA. 2011.** Systematic review of clinical trials on dietary interventions to prevent excessive weight gain during pregnancy among normal weight, overweight and obese women. *BMC Pregnancy and Childbirth* 11: 81 [http://www.biomedcentral.com/1471-2393/11/81](http://dx.doi.org/10.1038/nature05414).

**Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JJ. 2008.** Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 3: 213-223 DOI 10.1016/j.chom.2008.02.015.

**Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JJ. 2006.** An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444: 1027-1031 doi:10.1038/nature05414.

**Vemuri R, Gundamaraju R, Eri R. 2017.** Role of Lactic Acid Probiotic Bacteria in IBD. *Current Pharmaceutical Design* 23: 2352-2355 DOI: [10.2174/1381612823666170207100025](http://dx.doi.org/10.2174/1381612823666170207100025).

**Wang J, Tang H, Zhang C, Zhao Y, Derrien M, Rocher E, van-Hylckama Vlieg JE, Strissel K, Zhao L, Obin M, Shen J. 2015.** Modulation of gut microbiota during probiotic-mediated attenuation of metabolic syndrome in high fat diet-fed mice. *The ISME Journal* 9: 1-15 doi:10.1038/ismej.2014.99.

**WGO. 2017.** World Gastroenterology Organisation Global Guidelines - Probiotics and prebiotics. [http://www.worldgastroenterology.org/UserFiles/file/guidelines/probiotics-and-](http://www.worldgastroenterology.org/UserFiles/file/guidelines/probiotics-and-).
Woodard GA, Encarnacion B, Downey JR, Peraza J, Chong K, Hernandez-Boussard T, Morton JM. 2009. Probiotics improve outcomes after Roux-en-Y gastric bypass surgery: a prospective randomized trial. *Journal of Gastrointestinal Surgery* **13**: 1198-204  DOI 10.1007/s11605-009-0891-x.

Yanovski SZ, Yanovski JA. 2014. Long-term drug treatment for obesity: a systematic and clinical review. *JAMA* **311**: 74–86.

**Captions for Figures**

**Figure 1** (A) Body weight after 48 days, (B, C, D) and increase over the 48-day period; (E) body weight gain after 48 days, and (F) daily feed consumption of each group. LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *L. sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^9$ (medium-dose, M) and $1 \times 10^8$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.

**Figure 2** Serum biomarkers of each experimental group showing (A) triglycerides, (B) glucose, (C) total cholesterol, (D) high density lipoprotein (HDL), (E) low density lipoprotein (LDL) and (F) aspartate transaminase (AST). LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *L. sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^9$ (medium-dose, M) and $1 \times 10^8$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.

**Figure 3** Organ weights of each experimental group showing (A) epididymal adipose tissue, (B) mesenteric adipose tissue, (C) subcutaneous adipose tissue (D) brown adipose tissue, (E) liver
and (F) femoral muscle. LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CILS03 denote the three \textit{L. sakei} strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^{9}$ (medium-dose, M) and $1 \times 10^{8}$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.
Table 1 (on next page)

Diet composition of the low-fat (LFD) and high-fat (HFD) diets used in this study

Diet composition of the low-fat (LFD) and high-fat (HFD) diets used in this study (A) Low fat diet (Purina Laboratory Rodent Diet 38057); (B) High fat diet (Research Diets D12492).
Table 1  Diet composition of the low-fat (LFD) and high-fat (HFD) diets used in this study

(A) Low fat diet (Purina Laboratory Rodent Diet 38057); (B) High fat diet (Research Diets D12492).

A.

| Calories (%) | Ingredients | Protein (%) | Fat (%) | Fiber (%) | Minerals (%) | Vitamins (%) |
|--------------|-------------|-------------|---------|-----------|--------------|--------------|
| Fat          | 12.41%      | Arginine (1.26) | Glycine (0.87) | Linoleic Acid (1.10) | Ash (7.25) | Vitamins A, D3, E, K, Riboflavin, Niacin Others |
|              | Carbohydrate 63.07% | Isoleucine (0.82) | Leucine (1.47) | Lysine (1.01) | Linolenic Acid (0.12) | Crude fiber |
|              |              | Phenylalanine (0.98) | Valine (0.91) | Others | Arachidonic Acid (0.02) | Phosphorus (0.62) |
| Protein      | 24.52%      | Others | Others | Others | Omega-3 Fatty Acids (1.11) | Potassium (0.82) |
| Total        | 100%        | 20         | 4.5     | 3.7       |              |              |

B.

| Calories (kcal%) | Ingredients (g) |
|------------------|-----------------|
| Fat              | Casein, 80 Mesh (200) |
|                  | L-Cystine (3)    |
|                  | Maltodextrin 10 (125) |
|                  | Sucrose (68.8)   |
|                  | Cellulose, BW 200 (50) |
|                  | Soybean Oil (25) |
|                  | Lard (245)       |
| Carbohydrate     | Mineral Mix, S10026 (10) |
|                  | DiCalcium Phosphate (13) |
|                  | Calcium Carbonate (5.5) |
|                  | Potassium Citrate.1H2O (16.5) |
|                  | Vitamin Mix, V10001 (10) |
| Protein          | Choline Bitartrate (2) |
|                  | FD&Blue Dye #1 (0.05) |
| Total | 100%  | 773.85 |
Table 2

Study design and animal treatments based on a high-fat (HFD) and low-fat diet (LFD).

Study design and animal treatments based on a high-fat (HFD) and low-fat diet (LFD). LFD, low-fat diet (negative control); HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *Lactobacillus sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/ml (high-dose, H), $1 \times 10^{9}$ (medium-dose, M) and $1 \times 10^{8}$ CFU/ml (low-dose, L).
Table 2  Study design and animal treatments based on a high-fat (HFD) and low-fat diet (LFD).

LFD, low-fat diet (negative control); HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *Lactobacillus sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/ml (high-dose, H), $1 \times 10^9$ (medium-dose, M) and $1 \times 10^8$ CFU/mL (low-dose, L).

| Group | Feed type | Treatment |
|-------|-----------|-----------|
| LFD   | LFD       | 300 μL PBS (non-obese control) |
| HFD   | HFD       | 300 μL PBS (obese control) |
| Orlistat | HFD     | 40mg/kg suspended in 300 μl PBS |
| CJB38 L | HFD     | $1 \times 10^8$ CFU/day of *L. sakei* L338 suspended in 300 μL PBS |
| CJB38 M | HFD     | $1 \times 10^9$ CFU/day of *L. sakei* L338 suspended in 300 μL PBS |
| CJB38 H | HFD     | $1 \times 10^{10}$ CFU/day of *L. sakei* L338 suspended in 300 μL PBS |
| CJB46 L | HFD     | $1 \times 10^8$ CFU/day of *L. sakei* L446 suspended in 300 μL PBS |
| CJB46 M | HFD     | $1 \times 10^9$ CFU/day of *L. sakei* L446 suspended in 300 μL PBS |
| CJB46 H | HFD     | $1 \times 10^{10}$ CFU/day of *L. sakei* L446 suspended in 300 μL PBS |
| CJLS03 L | HFD     | $1 \times 10^8$ CFU/day of *L. sakei* LS03 suspended in 300 μL PBS |
| CJLS03 M | HFD     | $1 \times 10^9$ CFU/day of *L. sakei* LS03 suspended in 300 μL PBS |
| CJLS03 H | HFD     | $1 \times 10^{10}$ CFU/day of *L. sakei* LS03 suspended in 300 μL PBS |
Figure 1

(A) Body weight after 48 days, (B, C, D) and increase over the 48-day period; (E) body weight gain after 48 days, and (F) daily feed consumption of each group.

(A) Body weight after 48 days, (B, C, D) and increase over the 48-day period; (E) body weight gain after 48 days, and (F) daily feed consumption of each group. LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *L. sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^{9}$ (medium-dose, M) and $1 \times 10^{8}$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.
Figure 2

Serum biomarkers of each experimental group showing (A) triglycerides, (B) glucose, (C) total cholesterol, (D) high density lipoprotein (HDL), (E) low density lipoprotein (LDL) and (F) aspartate transaminase (AST).

Serum biomarkers of each experimental group showing (A) triglycerides, (B) glucose, (C) total cholesterol, (D) high density lipoprotein (HDL), (E) low density lipoprotein (LDL) and (F) aspartate transaminase (AST). LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *L. sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^9$ (medium-dose, M) and $1 \times 10^8$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.
Figure 3

Organ weights of each experimental group showing (A) epididymal adipose tissue, (B) mesenteric adipose tissue, (C) subcutaneous adipose tissue (D) brown adipose tissue, (E) liver and (F) femoral muscle.

Organ weights of each experimental group showing (A) epididymal adipose tissue, (B) mesenteric adipose tissue, (C) subcutaneous adipose tissue (D) brown adipose tissue, (E) liver and (F) femoral muscle. LFD, low-fat diet; HFD, high-fat diet; CJB38, CJB46 and CJLS03 denote the three *L. sakei* strains; the three dose levels of each strain administered together with the HFD were $1 \times 10^{10}$ CFU/mL (high-dose, H), $1 \times 10^{9}$ (medium-dose, M) and $1 \times 10^{8}$ CFU/mL (low-dose, L). The values for each index are expressed as the mean +/- SD (n = 10). Asterisks denote the level of significance compared to HFD as *: p<0.05, **: p<0.01 and ***: p<0.001.
