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The Effect of Lactobacillus genus on Weight Loss and Obesity Phenotype in Murine Species and Humans

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Title

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Abstract

The review attempts to determine the effects of *Lactobacillus* genus supplementation on weight and weight related factors. A literature review produced nine studies that met selection criteria. Of the nine articles included in the review, eight of them demonstrated weight loss among the study population after supplementation with *Lactobacillus* species. To initiate routine supplementation of *Lactobacillus* species in dietetic practice additional research is need to better understand mechanisms of action, optimal dosage, impact of varied macronutrient composition, and timeframe required for supplementation.

Introduction

Obesity continues to be a health concern not only in the United States, but worldwide. In just over three decades, the prevalence of obesity has nearly doubled worldwide with 11% of men and 15% of women aged 18 and over being classified as obese in 2014.[1] It is well established that obesity is impacted by genetic, lifestyle, and socioeconomic factors; however there appears to be an association between the gut microbiome and weight. The human intestinal microflora represents a complex ecosystem that is composed of trillions of microorganisms that function in host metabolism.[2] The association between the intestinal microflora and host metabolism was first discovered when an obese phenotype was produced in lean mice species after being transplanted with the intestinal microflora from obese mice.[4] There are several proposed mechanisms by which the intestinal microflora may impact body weight, including the efficiency of energy extraction, modulation of energy intake and metabolism, and regulation of satiety hormones and gut motility.[2] As such, there is increasing interest in the use of probiotics to modify weight in obese individuals. Probiotics are living microorganisms that when consumed have the ability to confer a health benefit.[3] Bacterial species belonging to the genus Lactobacillus and Bifidobacterium have been among the most commonly investigated probiotics in association with changes in adiposity.

There are in general four theories on potential mechanisms through which *Lactobacillus* species may influence obesity phenotype, including: (1)The intestinal microflora of obese subjects are more efficient at extracting energy from a given diet when compared to the intestinal microflora of lean individuals, thus leading to increased energy storage and adiposity.[4] (2) The intestinal microflora produce short chain fatty acids from fermentation of indigestible polysaccharides. The SCFA such as acetate, butyrate, and propionate function as both energy substrates and regulators of satiety and food intake. SCFA activate G-protein-coupled receptors GPR41 and GPR3 on intestinal epithelial cells and consequentially stimulate peptide YY and glucagon-like peptide (GLP)-1 secretion. These hormones are responsible for suppressing gut motility, extending transit time and thus allowing for greater nutrient absorption. (3) Conjugated linoleic acid (CLA) includes a mixture of linoleic acid isomers with conjugated double bonds that are a result of incomplete biohydrogenation of the unsaturated fatty acid linoleic acid. Humans are incapable of producing CLA from linoleic acid, thus the amount of CLA in human adipose tissue is directly related to the consumption of dietary CLA. Dietary sources of CLA include beef and dairy products such as milk fat, cheese, yogurt, and plant oil. It has been suggested that CLA may provide beneficial anti-carcinogenic activity, anti-arterogenic activity, ability to reduce catabolic effects of immune stimulation, and ability to reduce body fat. Synthesis of CLA which has anti-obesity properties.[2] (4) The inhibition of pro-inflammatory CCL2 and TNF-alpha are largely responsible for the anti-obesity effect of lactobacillus.[7] Research has suggested that a deficiency in the CCL2-CCR2 axis results in a reduction in visceral adipose tissue mass. Consequentially, reduced up-regulation of CCL2 and CCR2, as well as their regulator TNF-alpha may be proposed as a mechanism responsible for the reduction in visceral adipose tissue mass after consumption of lactobacillus. Of additional note inflammation in the adipose tissue is also closely related to the regulation of inflammation in the intestine.

Methods

The literature review was initiated by using search engines Ebscohost and CINAHL and keywords obesity and lactobacillus. Results were narrowed by selecting studies published between 2014 and 2019 in peer-review journals. There were not limitations with regards to study design; however study topic was limited to change in obesity phenotypes. Those studies that did not include assessment of changes in obesity phenotype were excluded from the review. Thus this review does not address the effect of *Lactobacillus* species on obesity-related factors such as hyperlipidemia and insulin resistance. Duplicates were manually identified and eliminated. All human studies included adult subjects, greater than 18-years of age and were classified as overweight or obese according to BMI. All articles obtained were written in the English language.

Discussion

Lactobacillus Rhamnosus

In the study conducted by Lee, et al. mice fed *L. rhamnosus* demonstrated decreased weight gain notable to the epididymal white adipose tissue despite lack of variance in energy intake compared to control group.[4] This effect is thought to be a result of the continuous production of CLA after colonization in the gut. Previous studies have consistently supported that idea that CLA supplementation induces a significant decrease in body fat deposition without significant reduction in caloric intake. Fat deposition changes are thought to be related to decrease in the number of fat cells rather than changes in the cell size. While beneficial effects on body weight were observed in subjects fed Lactobacillus rhamnosus PL60, there did not appear to be any dose dependent effect between those that received 1 x 10^7 (PL60L) or those that received $1x10^9$ (PL60H) CFU daily, despite the presence of serum CLA in the group receiving $1x10^9$. Longevity of the study is only 8 weeks; however reduction in body weight with administration of both PL60H and PL60L became narrower over the study period, suggesting that the dose used was sufficient to convey a maximal effect. Additionally, this study does not explore that need for continued supplementation or the required dose of PL60 to maintain weight loss benefits.

Similarly, the double-blind, placebo-controlled, randomized trial by Sanchez et al., conveys similar benefits on weight loss of the *Lactobacillus rhamosus* species only in the human population.[5] Over the course of 24 weeks study subjects consumed two capsules per day of either a placebo or *Lactobacillus rhamnosus* CGMCC1.3724 (LPR) formulation which contained 1.6 x 10^8 colon-forming units of LPR per capsule with oligofructose and inulin while adhering to a moderate energy restriction for 12-weeks (500 calories/day), followed by 12-weeks of maintenance. The trial found no significant change in weight loss during the energy-restriction or after the weight maintenance period. There was also no significant difference in fat mass. There were however significant variances when evaluating treatment x sex interaction. Specifically, during phase 1, there were more significant reductions in body weight and fat mass in the LPR-treated women than in the placebo-treated women. Additionally, at the end of phase 2 reductions in body weight and fat mass were more pronounced in the LPR-treatment women than in the placebo-treated women. Body weight and fat mass were not significantly affected by treatment in men. There were no significant differences on metabolic and inflammatory plasma markers during the trial period with the exception of leptin. At week 24, there was a greater decrease in fasting leptin concentrations among both men and women in the LPR group when compared with the placebo group. This suggests that there is a change in weight or metabolism as it is thought that leptin levels are associated with reduced energy stores and brain control of eating behaviors.[6]

Traditionally, leptin levels decrease during weight loss and encourage feeding, reduction in energy expenditure, and promotion of weight regain. Thus the there remains the question of longevity and ability for *Lactobacillus rhamnosus* to continue to suppress appetite despite weight loss and reduced leptin levels. While the study accounted for caloric intake and capped amount of probiotic-enriched products, it did not control for other lifestyle factors that may impact weight such distribution of macronutrients, intake of fiber, sugar, and fluids, sleep pattern, genetics, and exercise. Interestingly, the study did account for gastrointestinal factors that may affect the viability and efficacy of probiotic supplement capsules and added oligosaccharide and polysaccharide mixture to accommodate. Both oligosaccharides and polysaccharide are prebiotics which may exhibit there own selective benefits or enhance probiotic effects; however the study suggests that they did not likely have any independent effect on the LPR group.

Both the study conducted by Lee et al. and Sanchez et al. suggest a positive benefit to *Lactobacillus rhamnosus* on weight status. The Lee et al. trial did not differentiate between male and female species; however the Sanchez et al. trial suggested that supplementation with the *Lactobacillus rhamnosus* species benefits only women in the timeframe allotted, which provide strong evidence of the benefit of LPR supplementation.

Lactobacillus gasseri

The study included C57BL/6 mice that were divided into three groups: those fed a 5%-fat diet, 10%-fat diet, and 10%-fat diet containing the probiotic LG2055 (10% fat-LG).[7] The mice were allowed free access to their respective diet for 24 weeks. Weight, fat tissue mass, liver fat content, and inflammatory genes in adipose tissue, and lipogenic and lipolytic genes in the liver were then assessed. As expected, the mice fed the 10%-fat group had a significantly higher energy intake compared with the group fed a 5%-fat diet. Initial body weights were similar among the groups; however the final body weight in the 10%-fat group was significantly higher compared to the 5% fat group. The 10% fat-LG group demonstrated a significantly lower final body weight compared with the 10% fat group despite having similar energy intake. Additionally, the 10% fat-LG group had significantly lower relative weight of the retroperitoneal fat and epididymal compared with the 10% fat group. Triglyceride levels in the liver were significantly elevated in the 10% fat group compared with the 5% fat group and generally lower in the 10% fat-LG group compared with the 10% fat group. Additionally, the epididymal adipose tissue of the 10% fat group demonstrated higher expression levels of pro-inflammatory genes and adipocytokine gene, leptin when compared with the 5% fat group. In the liver, the 10% fat group offered higher expression of lipogenic genes, such as acetyl CoA carboxylase 1, fatty acid synthase, and sterol regulatory element-binding protein 1 compared with the 5% fat group and the 10% fat-LG group demonstrated levels similar to the 5% fat group. The 10% fat-LG group also demonstrated reduced levels of tumor necrosis factor alpha when compared with the 10% fat group. The results suggest that the inhibition of pro-inflammatory CCL2 and TNF-alpha are largely responsible for the anti-obesity effect of lactobacillus. Of additional noted, the expression of FAS, which is lipogenic, decreased in the 10% fat-LG group compared with the 10% fat group. In summation, the LG2055 is thought to decrease lipogenesis and increased lipolysis in the liver; however the results in this study were not significant.

The results may have been impacted by the type of fat used in the study groups. The 10% fat diet was comprised of lard compared with the 5% fat group, which utilized corn oil. The corn oil contains less saturated fatty acids than the lard. The higher concentration of linoleic acid in the corn oil may lead to intestinal inflammation which may in turn obscure the significance of the pro-inflammatory effects caused by higher calorie and fat consumption in the 10% fat groups. Additionally the long study period may have allowed for the intestine to adapt to low-grade inflammation, thus it may be more beneficial to assay pro-inflammatory markers at periodic intervals throughout the study.

Kadooka et al. conducted a multi-center, double-blind, randomized, placebo-controlled trial that included 87 generally healthy adults.[8] The study period included a 4-week lead-in period, followed by a 12-week consumption period in which subjects consumed either the fermented milk product containing 10^8 colon-forming units (CFU) of LG2055 or a control fermented milk-lacking LG2055. Subjects consumed 100 g twice a day of the active or control fermented milk for 12 weeks. Despite consistency in activity level, macronutrient composition, and total calorie intake among test subjects, there was a significant decrease in the visceral, subcutaneous, and total fat areas in the active group between week 0 and week 12; however there were no significant changes noted among the control group. Additionally, the active group demonstrated significant decreases from week 0 in the following parameters at the stated time points: body weight at W8 and W12, BMI at W8 and W12, waist circumference at W8 and W12, hip circumference at W8 and W12, and waist-to-hip ratio at W8. Conversely, the control group did not show any significant decreases in any of the parameters.

The study period is not sufficient to demonstrate continued effects of the probiotic supplement, nor is it sufficient to allow for a plateau in the study results. Thus, it would beneficial to prolong the study period to determine the timeframe required to obtain optimal supplement results and the ability to persist beneficial results in weight and adipose tissue after a plateau is obtained among participants. The trial accounts for factors such as macronutrient intake, total calorie intake, and exercise; however it does not account for other factors such as stress, sleep, and genetics, which may impact study results. Lastly, the fermented milk contains beneficial components such as calcium and conjugated linoleic acid, which may also positively benefit weight and obesity status; however both calcium and conjugated linoleic acid were common to both groups and thus not likely to have contributed to any of the documented benefits.

After demonstrating favorable benefits of fermented milk with 10^8 colony-forming units (CFU) of *Lactobacillus gasseri* supplementation in individuals with obese tendencies, Kadooka et al. attempted to explore a potential minimum CFU required to yield positive changes in obesity phenotype in a multi-center, double-blind, parallel-group, randomized-control group that included 210 Japanese adults with significant visceral fat areas.[9] The study period involved a 4-week lead-in period, followed by a 12-week consumption period, and a 4-week post-consumption period. Subjects consumed one of three products: (1) fermented milk containing 10^7 CFUs, (2) fermented milk containing 10^6 CFUs, or fermented milk containing 0 CFUs, for 12 weeks while maintaining usual diet and exercise. There were no significant differences in abdominal subcutaneous fat areas; however visceral fat decreased significantly at weeks 8 and 12 from baseline in the active group. Additionally, BMI, waist, and hip circumferences were significantly reduced in both the 10^7 and 10^6 dose groups at week 8 and 12 from baseline; however at 4 weeks after the completion of consumption, the amount of changes in all measures in both the 10^7 and 10^6 dose groups became smaller than observed at week 12. Only fat mass demonstrated a significant decrease at week 12 both from baseline and against the control in both the 10^7 and 10^6 dose groups; however the significance from baseline disappeared at 4-weeks after complete consumption.

Contrary to previous studies, Kadooka demonstrates a peak of effects on BMI and waist and hip circumference. The study also suggests that without continued supplementation of the Lactobacillus product the achieved benefits cannot be maintained for more than four weeks. All three products were identical with the exception of the Lactobacillus, thus nutrients such as calcium and fat content would have contributed that same effect on all subject groups. Consequentially, it can be assumed that the results of the study are a result of the Lactobacillus. Lastly, similar to other studies included the review, there is no control for other various lifestyle factors including diet, exercise, sleep, stress, and inactivity that may influence weight related factors.

Lactobacillus acidophilus

Arora et al. confused a study that included 24 male C57BL/6 mice were first acclimated to a normal chow diet for one week.[10] Following the acclimation period, mice were randomized according to weight and divided into two groups: control dahi and probiotic dahi. The viable counts of viable counts of *L. acidophilus* in the probiotic dahi were in the range of 5×10^7 to 9×10^7 CFU/ml. The only difference between the control and the test samples was the addition of *L. acidophilus.* Both groups were fed a 21% high-fat diet and their respective dahi products for eight weeks. The probiotic group had no significant changes in body weight or food intake. Additionally, there were no significant differences in visceral or subcutaneous fat deposits by MRI between the control and the probiotic dahi-fed mice. There was however a significant increase in the fecal *Bifidobacterium* counts in the control and probiotic-fed groups at week 8. Additionally, the control group experienced a significant decrease in *Lactobacillus-Enterococcus* at week 8 compared with week 1; however there was no significant difference observed in total bacteria. There observations may suggest that *Lactobacillus acidophilus* species was ineffective in colonizing the intestinal walls and proliferating in the intestinal environment under high dietary fat conditions. It may have also been that the dose of *Lactobacillus acidophilus* was insufficient to encourage colonization and proliferation.

While Arora et al. did not demonstrate any changes to obesity related factors with supplementation of *Lactobacillus acidophilus,* there were a number of study flaws including the small population size limited to just males and lack of information regarding weight status. In the event that all mice were of normal weight and adiposity, it would be difficult to demonstrate changes to weight and adipose tissue with supplementation of *Lactobacillus acidophilus.* Additionally, the range of the viable *Lactobacillus acidophilus* was too large to determine sufficient dose required to produce desired results. The study would be difficult to reproduce with the variance in CFUs amount in samples provided to subjects. While the study used a medium that already contained beneficial bacteria, *Lactococcus,* the results are likely a result of the

Lactobacillus acidophilus as both the control and the test products were the same with the exception of the addition of the *Lactobacillus acidophilus* in the test product. Additional research is necessary to further investigate potential benefits of *Lactobacillus acidophilus* on weight related factors.

Lactobacillus reuteri

The study included 10 rats that were fed a normal diet and placebo (distilled water).[10] The remaining 30 rats were fed a high-energy diet (HED) and were then randomly assigned to one of three groups, including the HE group, the 1X group (HED and 2.1 x 10^9 CFU/kg/day of *L. reuteri* 263) and the 5X group (HED and 1.05 x 10^{10} CFU/kg/day of *L. reuteri*). Daily food and water intake, weekly weights adipose tissue, biochemical profiles, adipocyte, and oxygen consumption rate were assessed. The food energy intake was higher in the HED-fed rats than the normal-fed diets and subsequently the average body weight of the HE group increased the most. The body weight of the 1X group was slightly higher than the 5X group. The control group had the smallest increase in body weight. The HE group demonstrated significantly higher levels of fasting blood sugars, triglycerides, total cholesterol, and LDL compared to the other groups. Additionally, the HE group also had the lowest HDL compared to either of the other groups. The level of the inflammatory factors IL-6 and TNF-alpha were 2 and 1.5 times higher in the HE group, while the 1X and 5X group had significantly higher levels than the control group. As expected, the control group had the lowest percentage body fat; however the HED-derived high percentage body fat was modulated with administration of daily doses of *L. reuteri* 263. The HE group also had the highest weight for EFPs, renal fat pads, and mesenteric fat pads, followed by the 1X, 5X, and C groups, respectively. Lastly, the 5X group had the highest bioenergetic health index (BHI), approximately 1.4 times that of the C group and 2.7 time the HE group. The potential benefit effects of *L. reuteri* may be related to the upregulation of mitochondrial respiration in white adipose tissue (WAT) following oral *L. reuteri* 263.

The study sample was limited to 8-week old male mice with no evidence of obesity at baseline. Of the studies collected, Chen et al. was the only to assess WAT and the oxygen consumption of WAT, which plays an importance role in anti-obesity. WAT is typically viewed as an energy-storing tissue, but has demonstrated the ability to dissipate energy and upregulate energy expediting genes such as *Ucp 1, Ucp3, Cpt1α,* and *Cidea.* The variance in WAT among study groups and the increase in energy expediting genes in the 5X group suggest that energy remodeling of WAT may improve obesity. *L. reuteri* may induce the WAT browning pathway by regulating cytokines, gut peptides, and neurotransmitters in the gut to influence the development and behavior of the brain. In turn the brain can increase the browning of WAT by activating the sympathetic nervous system innervation. This process is referred to as the gut-brain axis.

In Summary, *Lactobacillus rhamosus and Lactobacillus gasseri* appear to be the most prominent *Lactobacillus* genus to benefit weight status among both rats and humans. Supplementation with *Lactobacillus rhamosus* decreased weight in rats and yielded significant reductions in weight and fat mass among only women.[5,6] Both men and women experienced a decrease in fasting leptin levels, which may suggest a possible mechanism behind the benefit of *Lactobacillus rhamosus* on weight related factors.[6] *Lactobacillus gasseri* supplementation appeared to protect against weight gain and accumulation of epididymis and retroperitoneal fat stores despite energy intakes similar to those receiving an identical diet.[7] Additionally, the supplementation of *Lactobacillus gasseri* discouraged an increase in lipogenic genes in rats. In the human population, *Lactobacillus gasseri* supplementation produced significant decreases in visceral, subcutaneous, and total fat areas, as well as significant decreases in total body weight, waist-to-hip ratio, hip circumference, and waist circumference at various points throughout the study in the absence of dietary or lifestyle changes.[8] Kadooka et al. also demonstrated that 10^6 CFUs is sufficient to yield positive results. *Lactobacillus reuteri* appears to protect against fat accumulation and weight gain in rats; however those subjects on a regular diet without probiotic supplementations maintained the lowest weight and fat deposits.[11] The species *Lactobacillus acidophilus* did no produce any benefits with regard to weight; however additional studies are necessary to further investigate potential to influence weight.

While the review suggests that the *Lactobacillus* genus has great potential to moderate weight, fat deposition, and other weight related factors, there are a number of shortcomings of the reviewed studies. Off the studies, with the exception of one, had a small population size consisting of less than 100 subjects. While all studies included a washout period, there were lifestyle factors such as calorie intake, exercise length and intensity, and amount and/or quality of sleep that were not controlled during the study period. The animal studies reviewed replicate the high-fat diet of a typical western USA diet with the average fat content of approximately 33%; however the studies do not reflect the high levels of refined sugars and oils that are often consumed regularly by most Americans.[12] Consequentially, the impact of varied nutrient intake may alter the results of conducted trials. Lastly, all of the studies included in the review lacked the ability to demonstrate the need for continued supplementation to sustain weight and fat deposit changes. Only a limited number of studies demonstrated a plateau with supplementation, thus suggesting that benefits were no optimized with the timeframe of the study. Understanding the impact and efficacy of *Lactobacillus* strains on weight would yield a valuable resource for Registered Dietitians working with overweight and obese individuals.

Conclusion

In conclusion, there appears to be an inverse relationship between supplementation with the *Lactobacillus* species, including *Lactobacillus gasseri, Lactobacillus rhamosus,* and *Lactobacillus reuteri* on weight status, fat accumulation, and weight related factors; however more research is necessary to determine mechanisms of action, optimal dosage, impact of varied macronutrient composition, and timeframe required for supplementation. Ongoing research regarding the *Lactobacillus* genus would increase opportunities for Registered Dietitians to impact weight management success in overweight and obese individuals. Further research may also unveil other information regarding the benefits of various species on the microbiome and subsequent health benefits and respective mechanisms. With a greater understanding of the microbiome and the impact of supplementation with the *Lactobacillus* genus and other probiotics, would further emphasis the necessity of dietitians as part of the healthcare team. Exploration of probiotics and the microbiome is a great opportunity for dietitians to encourage all individuals to explore the impact of all foods and nutrients on the diverse microbiome.

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