# Effect of *Lactobacillus reuteri* on the Prevention of Hypercholesterolemia in Mice

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## ABSTRACT

Administration of *Lactobacillus reuteri* CRL 1098 ($10^4$ cells/d) to mice for 7 d before inducing hypercholesterolemia (by feeding mice with a fat-enriched diet for the subsequent 7 d) was evaluated. At this low dose, *L. reuteri* was effective in preventing hypercholesterolemia in mice, producing a 17% increase in the ratio of high-density lipoprotein to low-density lipoprotein. Total cholesterol and triglycerides decreased by 22 and 33%, respectively, in the group that was not fed the lactobacilli. The hypocholesterolemic effect produced by *L. reuteri* CRL 1098 might be considered as indirect evidence of the permanency of the lactobacilli in the gut.
(**Key words:** *Lactobacillus reuteri*, hypocholesterolemia, probiotic)

**Abbreviation key: FD** = fat-enriched diet, **HDL** = high-density lipoprotein, **LDL** = low-density lipoprotein.

## INTRODUCTION

During the last few decades, numerous epidemiological, laboratory, and clinical studies have demonstrated a connection between high serum cholesterol and increased risk for atherosclerosis and coronary heart disease, the latter being a major cause of death in Western countries (1). Potential hypocholesterolemic pharmaceuticals and food products are continuously being developed to control hypercholesterolemia in humans (9, 16).

With the emergence of a more health-conscious society, the role of probiotic food products has gained attention from consumers and producers (13). In this respect, the ingestion of probiotic lactic acid bacteria might be a more natural way to decrease serum cholesterol in humans (2). In a previous work (19) the hypocholesterolemic effect of *Lactobacillus reuteri* CRL 1098 in hypercholesterolemic mice was obtained at a very low dose ($10^4$ cells/d). This dose is the lowest reported found to be effective in decreasing serum lipids without producing side effects, e.g., bacterial translocation (3). Rodas et al. (15) observed a hypocholesterolemic effect in pigs fed large doses of *L. acidophilus* (about $10^{12}$ cells/d) that were many times higher than the doses ($10^4$ cells/d) used in our previous study. Grunewald (8) found similar effects in rats that were fed large amounts of fermented dairy products, while Gilliland and Walker (7) reported no probiotic effect in a study with human subjects. These controversial results may be partially explained by the fact of use of unsuitable probiotic bacteria, which possibly justified the use of high doses ($10^6$ to $10^8$ viable cells) to ensure the probiotic effect in the host (10).

In the present study, mice were fed *L. reuteri* CRL 1098 before administration the enriched-fat diet to evaluate whether the probiotic at very low doses ($10^4$ cells/d) helps to prevent hypercholesterolemia. Total cholesterol, triglycerides, and the ratio of high-density lipoproteins (**HDL**) to low-density lipoproteins (**LDL**) were evaluated.

## MATERIALS AND METHODS

### Microorganism and Culture Conditions

The strain *L. reuteri* CRL 1098 used in this study was obtained from the culture collection of Centro de Referencia para Lactobacilos (CERELA, San Miguel de Tucumán, Argentina). The microorganism was cultured in MRS broth (4) at 37°C for 16 h; the cells were harvested by centrifugation (6000 × *g* for 10 min) and washed three times with a sterile saline solution. The cells were then resuspended in sterile 10% NDM.

### Mice and Feeding Procedure for Evaluating *L. reuteri* Effects

Swiss Albino mice weighing 25 g were obtained from the random-bred closed colony at CERELA. The mice

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**Table 1**. Diets fed to the different groups.

| Group | Diet |
|---|---|
| Control | Chow[1] and water (2 wk) |
| FDr[1] with *Lactobacillus reuteri* | Chow and *Lactobacillus reuteri* (1 wk)<br>Chow and milk cream-enriched NDM (1 wk) |
| FD without *Lactobacillus reuteri* | Chow and water (1 wk)<br>Chow and milk cream-enriched NDM (1 wk) |

[1]Chow = 32% protein, 5% fat, 2% fiber, and 60% nitrogen-free extract.

[2]FD = Fat-enriched diet.

were split into three experimental groups, each one consisting in 10 mice housed individually and maintained on a cycle of 12 h of light and 12 h of dark. All groups received a solid conventional diet (rodent chow: 32% protein, 5% fat, 2% fiber, and 60% nitrogen-free extract). One group (FDr) was fed for 7 d with *L. reuteri* at a concentration of $10^4$ cells/d per mouse. The viable cells of *L. reuteri* were suspended in NDM as before, and administered at 20% (vol/vol) in the drinking water. The other group (FD) received 20% (vol/vol) NDM in the drinking water (without *L. reuteri*) during the same time. At the final period of treatment, the hypercholesterolemia was induced in both groups FD with a fat-enriched diet as described previously (19), which was administered for the subsequent 7 d. The third group (control) received only the solid conventional diet and water for 15 d. The total fat content of the diet was 17.6% for FD groups and 6.7% for the Control group. The composition of the diets is given in Table 1.

After 2 wk, blood samples of 10 mice per group were drawn from the retroorbital venous plexus for determination of serum total cholesterol, HDL cholesterol, LDL cholesterol, and serum triglycerides. The cholesterol and triglycerides concentrations were determined enzymatically using an enzymatic reagent kit (Sigma Chemical Co., St. Louis, MO).

### Statistical Analysis

The following variables were taken into consideration: total cholesterol, ratio of HDL to LDL, and concentration of triglycerides. The means per mouse of each one of these variables and the differences among them for each treatment, were measured by ANOVA. The results obtained for each treatment group were evaluated in pairs by the honestly significant difference test of Tukey. The cluster analysis of the different groups was performed by K-means. Calculations were made with the software SYSTAT (17).

## RESULTS AND DISCUSSION

The fat-enriched diet fed to FD group increased the total cholesterol by 1.8 times, triglycerides by 1.6 times, and decreased the ratio HDL cholesterol to LDL cholesterol by 1.3 times compared with the values of the control group (Table 2). The increase in lipid concentration was significant ($P < 0.001$). However, no differences ($P > 0.05$) were obtained in the weight of spleen and liver in relation to the BW among groups throughout the study.

The 7-d dietary pretreatment of the FDr group with *L. reuteri* CRL 1098 decreased total cholesterol and triglycerides by 20 and 33% ($P < 0.001$), respectively, compared with the FD group that received milk in the drinking water without *L. reuteri*. A 17% increase in the ratio of HDL to LDL was also obtained for the group treated with the lactobacilli, probably indicating a lower amount of cholesterol bound to the LDL.

The cluster analysis of the total cholesterol, the ratio of HDL to LDL, and the triglycerides were performed for each mouse of each feeding group. These results determined the distribution of the mice into three groups, which was performed by K means procedure (17). Thus, group 1 included FD mice that were pretreated with *L. reuteri*, group 2 corresponded to the control group, and group 3 was the FD group that was not fed the lactobacilli. Euclidean distances among these groups are presented in Table 3. The data clearly show the relationship

**Table 2**. Effect of *Lactobacillus reuteri* on total cholesterol, triglycerides, and ratio of HDL to LDL suministered previously to a fat-enriched diet (n = 20).[1]

| | Groups | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | FDr with *L. reuteri* | | FD without *L. reuteri* | |
| | $\bar{X}$ | SD | $\bar{X}$ | SD | $\bar{X}$ | SD |
| Total cholesterol mg/dl | 67.4[a] | 6.9 | 96.2[a] | 4.5 | 120.5[b] | 3.5 |
| Triglycerides mg/dl | 85.7[a] | 14.5 | 87.8[a] | 9.9 | 140.1[b] | 11.0 |
| Ratio of HDL to LDL[1] | 1.5[a] | 0.2 | 1.4 | 0.3 | 1.2[b] | 0.1 |

[a,b]Means within a row with no common superscripts differ ($P < 0.05$).

[1]HDL = High-density lipoprotein; LDL = low-density lipoprotein.

that exists between groups 1 and 2 on the basis of the slight difference (1.25) between them. In contrast, both groups are separated from group 3 by 1.32 and 1.54, respectively.

Lactic acid bacteria and more generally "transiting microorganisms" can be considered as an original method to deliver active constituents to targets in the gastrointestinal tract. The destruction of ingested probiotics in the gut is mainly caused by acid in the stomach and bile in the intestine; survival depends on their intrinsic resistance but also on the host and on the product in which the probiotics are ingested. Previous assays put in evidence the high intrinsic resistance of *L. reuteri* to low pH (2 log units decrease in viability after 24 h at pH 2.0) and to the presence of bile salts (18) compared with the yogurt bacteria (5) and even with *L. acidophilus* (7). The high tolerance of *L. reuteri* to acid and bile (the main natural barrier to the entrance of exogenous microbiota into the gastrointestinal tract) would be related to its intestinal origin (6). In fact, Mitsouka (12) reported that this species was a major component of gut lactobacilli and the only *lactobacillus* known to be indigenous to a broad phylogenetic spectrum of hosts, including humans and all mammals and avian hosts examined to date.

Several trials have noted a decrease in serum cholesterol in animal and subjects (8, 11, 14). However, such an effect seems to persist as long as the probiotic supplement was fed; when it was stopped, cholesterol returned to presupplementation levels. A different situation was observed in our study with *L. reuteri* CRL 1098 as prophylactic dietary adjunct; the serum cholesterol level in lactobacillus-pretreated mice (FDr) increased only 38% compared with the FD group (82%) that did not receive *L. reuteri* (Table 2). These results show the effectiveness of *L. reuteri* as prophylactic in preventing hypercholesterolemia. In addition, the hypocholesterolemic effect observed when the treatment was stopped provides indirect evidence of the permanency of *L. reuteri* CRL 1098 in the gut.

**Table 3**. Euclidean distance between the treatment groups.

| Group[1] | 1 | 2 | 3 |
|---|---|---|---|
| 1 | 0.00 | 1.25 | 1.32 |
| 2 | 1.25 | 0.00 | 1.54 |
| 3 | 1.32 | 1.54 | 0.00 |

[1]1 = FDr group treated with *Lactobacillus reuteri*, 2 = control group, 3 = FD group without *Lactobacillus reuteri*. Groups were classified according to the K-means procedure. FD = fat-enriched diet.

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## REFERENCES

1 Barr, D. P., A. M. Russ, and H. A. Eder. 1951. Protein-lipid relationship in human plasma. II. In atherosclerosis and related conditions. Am. J. Med. 11:480–493.

2 Bazzare, T. L., S. Liu Wu, and J. M. Yuhas. 1983. Total and HDL-cholesterol concentrations following yogurt and calcium suplementation. Nutr. Rep. Int. 28:1225–1232.

3 Berg, R. D. 1979. Bacterial translocation from the intestines. Exp. Anim. 34:1–16.

4 De Man, J. C., M. Rogosa, and M. E. Sharpe. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. 23:130–135.

5 Conway, P. L., S. L. Gorbach, and B. R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. J. Dairy Sci. 70:1–12.

6 Dobrogoz, W. J., I. A. Casas, G. A. Pagano, T. L. Talarico, B. M. Sorberg, and M. Karlson. 1989. *Lactobacillus reuteri* and the enteric microbiota. Pages 283–292 *in* The Regulation and Protective Role of the Normal Microflora. R. Grubb, T. Midvedt, and E. Norin, ed. Macmillan, Ltd., London.

7 Gilliland, S. E., and D. K. Walker. 1990. Factors to consider when selecting a culture of *Lactobacillus acidophilus* as a dietary adjunct to produce a hipocholesterolemic effect in humans. J. Dairy Sci. 73:905–911.

8 Grunewald, K. K. 1982. Serum cholesterol levels in rats fed skim milk fermented by *Lactobacillus acidophilus*. J. Food Sci. 47:2078–2079.

9 McNamara, D. J., A. M. Lowell, and J. E. Sabb. 1989. Effect of yogurt intake on plasma lipid and lipoprotein levels in normolipidemic males. Atherosclerosis 79:167–171.

10 Mann, G. V. 1977. A factor in yogurt which lowers cholesterolemia in man. Atherosclerosis 26:335–340.

11 Massey, L. 1984. Effect of changing milk and yogurt consumption on human nutrient intake and serum lipoprotein. J. Dairy Sci. 67:255–262.

12 Mitsouka, T. 1992. The human gastrointestinal tract. Pages 159–172 *in* Lactic Acid Bacteria, Vol. I, The Lactic Acid Bacteria in Health and Disease. B.J.B. Wood, ed. Elsevier, New York.

13 Perdigón, G., M.E.B. de Jorrat, S. F. de Petrino, and M. Valverde de Budeguer. 1991. Effect of oral administration of *Lactobacillus casei* on various biological functions of the host. Food Agric. Immunol. 3:93–102.

14 Rao, D. R., C. B. Chawan, and S. R. Pulusani. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. J. Food Sci. 46:1339–1341.

15 Rodas, B. Z., S. E. Gilliland, and C. V. Maxwell. 1996. Hypocholesterolemic action of *Lactobacillus acidophilus* ATCC 43121 and calcium in swine with hypercholesterolemia induced by diet. J. Dairy Sci. 79:2121–2128.

16 Suckling, K. E., G. M. Benson, B. Bond, A. Gee, A. Glen, C. Haynes, and B. Jackson. 1991. Cholesterol lowering and bile acid excretion in the hamster with cholestyramine treatment. Atherosclerosis 89:183–190.

17 System for Statistics (SYSTAT). 1990. Wilkinson, ed. Evanston, IL.

18 Taranto, M. P., F. Sesma, A. P. Ruiz Holgado, and G. F. Valdez. 1997. Bile salts hydrolase plays a key role on cholesterol removal by *Lactobacillus reuteri*. Biotechnol. Lett. 9:245–247.

19 Taranto, M. P., M. Medici, G. Perdigón, A. P. Ruiz Holgado, and G. F. Valdez. 1998. Evidence for hypocholesterolemic effect of *Lactobacillus reuteri* in hypercholesterolemic mice. J. Dairy Sci. 81:2336–2340.