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Regulatory Effect of Probiotics on Cholesterol Levels—A Review of *In Vitro*, *In Vivo* and Human Studies.

Eddy E Owaga^{a,*}, and Masuku Sakhile KS^b.

^aSchool of Nutrition and Health Sciences, College of Public Health and Nutrition, Taipei Medical University, No. 250, Wu-Hsing St., Taipei 110, Taiwan.

^bDepartment of Community Health Nursing, Faculty of Health Sciences, University of Swaziland. P. O. Box 369, Mbabane, Swaziland.

ABSTRACT

Elevated blood cholesterol is an important risk factor of cardiovascular heart diseases. The modulatory role of probiotic bacteria on serum cholesterol levels has attracted much research interest in past years. Several probiotic studies show reduced levels of total serum cholesterol, low density lipoprotein and triglyceride. The suggested mechanisms include: enzymatic deconjugation of bile acids by bile-salt hydrolase, assimilation of cholesterol, co-precipitation of cholesterol with deconjugated bile, binding of cholesterol to cell walls, incorporation of cholesterol into the cellular membranes of probiotics during growth, conversion of cholesterol into coprostanol and production of short-chain fatty acids. Moreover, molecular studies have revealed a new mechanism that is potentially responsible for the cholesterol-reducing effects of probiotics. It has been observed that control of cholesterol is, at least partially, mediated by the bacterial down-regulation of Niemann-Pick C1-like 1 (NPC1L1) protein, which when weakly expressed in the small intestine, results in a marked reduction in intestinal absorption of cholesterol. The regulatory role of probiotics in cholesterol metabolism underscores the basis for the use of probiotic bacteria in managing hypercholesterolaemia. The aim of this review is to highlight the potential use of probiotic bacteria in managing hypercholesterolemia, with particular focus on the mechanisms by which such effects might be exerted.

Keywords: probiotics, cholesterol, Niemann-Pick C1-like 1, liver X receptors, lactobacilli

**Corresponding author*

INTRODUCTION

Several epidemiological and clinical studies indicate a positive correlation between elevated total serum cholesterol levels and risk of emergence of cardiovascular heart diseases (CVD) [1]. A reduction of even 1% in serum cholesterol concentrations has been shown to reduce the risk of CVD in human subjects by 2–3% [2]. Due to the increased mortality rates associated with CVD, expenses of drug therapy and side effects of such treatments, there is intense research interest in the dietary approach including probiotics towards the management of serum cholesterol and triglycerides levels. Probiotics are live micro-organisms, which when administered in adequate amounts confer a health benefit on the host. The most common species belong to the genera *Lactobacillus* and *Bifidobacterium* [3].

An early study done by Mann and Spoerry, amongst men from a pastoral community in Kenya showed reduced serum cholesterol after consumption of large amounts of milk fermented with a wild *Lactobacillus* strain [4]. Some of the hypothesized mechanisms for the hypocholesterolemic effect include enzymatic deconjugation of bile acids by bile-salt hydrolase, incorporation of cholesterol into the cellular membranes of probiotics during growth and production of short-chain fatty acids [5]. The most recent proposed mechanism involves bacterial modulation of the absorption of dietary cholesterol and the reabsorption of biliary cholesterol in the small intestine [6]. The molecular mechanism by which intestinal cholesterol absorption occurs has recently been elucidated. The Niemann-Pick C1-like 1 (NPC1L1) protein has been identified as a key player in cholesterol absorption, and it is a promising target for cholesterol-lowering approaches [7]. The potential hypocholesterolemic effect of fermented milk products containing lactobacilli and/or bifidobacteria has been investigated in various models as deliberated below.

Probiotic effect on cholesterol levels - *in vitro* model

Several *in vitro* studies have demonstrated the cholesterol reducing ability of the *Lactobacilli* [8-10]. NPC1L1, which belong to the nuclear receptor superfamily, has been identified as a novel target gene of the liver X receptors (LXR α and β) that are crucial regulators of cholesterol homeostasis [11]. The activation of LXR reduces whole-body cholesterol and decreases atherosclerosis. In a study conducted by Huang and Zheng, [10], using Caco-2 cell line, it was showed that *L. acidophilus* ATCC 4356 and *L. rhamnosus* GG were better inhibitors of NPC1L1 than the *Bifidobacterium B. lactis* 12, which demonstrate distinctions among probiotic strains. In the same study, the cell supernatant of *L. acidophilus* ATCC 4356 grown without Caco-2 cells reduced NPC1L1 expression. These results indicate that soluble factors generated from bacteria can suppress NPC1L1 expression independent of contact between the bacteria and cells. Other authors have also reported that soluble factors secreted by probiotics can sufficiently modulate host cell functions [12]. These molecular findings on probiotic effect on cholesterol reduction, suggest that it may be possible to modify NPC1L1, a central player in cholesterol homeostasis, by manipulating the gut microbiota. Hence, probiotics such as *L. acidophilus* ATCC 4356, possibly via LXR signalling, could be a useful tool in cholesterol-lowering treatments.

Probiotic effect on cholesterol levels - *in vivo* animal model

Several *in vivo* studies have demonstrated that ingestion of *Lactobacillus* bacteria results in a reduced serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TAG) concentrations of rats fed a high-cholesterol diet [13, 14]. It has also been shown that NPC1L1 protein is responsible for intestinal cholesterol absorption [7]. Mice lacking NPC1L1 display a substantial reduction in cholesterol absorption and are resistant to both diet-induced hypercholesterolaemia and apoE deficiency-induced atherosclerosis [15]. According to a study conducted by Huang et al., [16], substantial reductions in cholesterol and TAG concentrations were observed in the liver and blood of rats in the *L. acidophilus* ATCC 4356-fed group, suggesting that the cholesterol concentration was actually reduced, and not merely re-distributed between the blood and liver. However, some researchers have reported no hypocholesterolemic effects from lactic acid bacteria in mice models [17]. Nevertheless, these conflicting results may be due to the different properties of the cultures used (e.g. acid and bile tolerance), amount of bacteria ingested, the cholesterol content of the diet under study, the animal used and the length of the feeding period [18, 19]. Although other animal studies have implicated the incorporation of cholesterol into cellular membranes or the deconjugation of bile salts during bacterial growth as the underlying mechanism for the hypocholesterolemic effects, Huang *et al.*, [16], indicated that *L. acidophilus* ATCC 4356 is able to reduce cholesterol absorption by inhibiting NPC1L1 mRNA transcription in the small intestine in rats. The expression level of NPC1L1 along the length of the small intestine has been correlated with the efficiency of cholesterol absorption, with the highest level being found in the proximal intestine (duodenum and jejunum) and lower expression being found in the distal intestine (ileum) [16, 20].

Probiotic effect on cholesterol levels – human studies

In contrast to *in vitro* and *in vivo* studies, clinical trials on the cholesterol-lowering properties of fermented dairy products are not conclusive [21]. Several authors have reported the reduction effect of TC, LDL-C, TAG by probiotics in human subjects [4]. In a study by Schaafsma et al. [22], a substantial reduction of serum cholesterol concentrations was observed after consumption of fermented milk containing *L. acidophilus* for 21 days. Intake of a milk product fermented with *Enterococcus faecium* and two strains of *S. thermophiles* also resulted in a significant reduction in LDL-cholesterol concentration after 6 weeks [23]. On the contrary, other authors have indicated no significant effect attributed to probiotic yoghurt when compared to control. In a study by Sadrzadeh-Yeganeh et al., [24], no statistically significant difference were found on the lipid profile in healthy women between probiotic yoghurt containing *L. acidophilus* La5 and *B. lactis* Bb12 and conventional yoghurt group. Nevertheless, these contradictory outcome have been attributed to different experimental designs, different strains of lactic bacteria, and intakes of fermented milk as a confounding factor, thereby leading to difficulty in evaluating the results. In the human studies, several mechanisms could be responsible for the hypocholesterolemic effect of probiotics, including: suppression of NPC1L1 expression hence modulates the absorption, production of the short chain free fatty acids in the gut, production of sphingolipids from bacterial cell membrane and deconjugation of the bile salts in the small intestine [25].

CONCLUSION

Probiotic have been widely assessed for their effects on lipid profiles such as total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides. In order to justify the cholesterol-lowering effect exhibited by various strains of probiotics, researchers have endeavored to investigate the mechanisms responsible for the probiotic hypocholesterolemic effect using various models. The additional understanding of the novel NPC1L1 protein is vital target in the design of probiotic approaches in the prevention or management of hypercholesterolemia.

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