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Cholesterol Removal Effect and Bile Salt Hydrolase by Probiotic Lactic Acid Bacteria

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ABSTRACT: Dietary habit change toward lipid-rich foods in recent years has resulted in risks for cardiovascular diseases. Serum cholesterol level is commonly recognized as an important factor in disease development. In this case, one of the most significant groups of probiotic organisms are the lactic acid bacteria, probiotics are live microorganisms that promote health benefits upon consumption, they could be used as alternative supplements to exert health benefits, including cholesterol-lowering effects on humans. However, little information is available on the effective dosage of probiotics needed to exert hypocholesterolemic effects. Both probiotics and prebiotics have been suggested to reduce cholesterol via various mechanisms. Though, more clinical evidence is needed to strengthen these proposals. The objective of this article was to review existing literature concerning the effects and mechanisms of action of probiotics on serum cholesterol concentrations.

Key words: probiotic, cholesterol-lowering, bile, bile salt hydrolase.

INTRODUCTION

Nowadays, people are faced with a lot of health problems caused by their lifestyle like heart problems. The WHO has predicted that by 2030, cardiovascular diseases will stay the leading causes of death, affecting about 23.6 million people around the World. It was reported that hypercholesterolemia contributed to 45% of heart attacks in Western Europe and 35% of heart attacks in Central and Eastern Europe from 1999 to 2003. Like many other countries, in Iran the coronary heart disease (CHD) associated with hyperlipidemia are considered as the main cause of death. In fact, the risk of heart attack is three times higher in those with hypercholesterolemia compared to those who have normal blood lipid profiles. Also each raise in the serum cholesterol by 1% results in 2.3% increase in the risk of CHD. The WHO delineated that unhealthy diets such as those high in fat, salt and free sugar, and low in complex carbohydrates, fruits and vegetables, lead to increased risk of cardiovascular diseases.

Furthermore, cholesterol (5-cholesten-3-ol) has been linked with the development of colon cancer and the potent angiotoxic effects of several cholesterol oxides have led researchers to hypothesize a likely role for them in cardiovascular diseases. Recent modalities for lowering blood cholesterol include dietary management, behavior modification, regular exercise, and drug therapy. Pharmacological agents that successfully reduce cholesterol levels are available for the treatment of high cholesterol; though, they are expensive and are known to have severe side effects. Probiotic Lactic acid bacteria (LAB) with active bile salt hydrolase (BSH) or products containing them have been suggested to lower cholesterol levels through interaction with host bile salt metabolism.

Probiotics have a long history of human use, and cultured dairy products, for example, are traditionally consumed in several parts of the world. Probiotics are defined as 'live microbial supplement that beneficially affects the host by improving its intestinal microbial balance'.

Probiotic: The concept of probiotics evolved around 1900, when Nobel Prize-winning Elie Metchnikoff hypothesized that the long, healthy lives of Bulgarian peasants were the result of their consumption of fermented milk products and later he was convinced that yogurt contained the organisms necessary to protect the intestine from the damaging effects of other harmful bacteria. Micro-organisms associated with health benefits in vivo include members of the Lactobacillus and Bifidobacterium genera, although streptococci, enterococci, lactococci, bacilli and fungi such as *Saccharomyces* spp. and *Aspergillus* spp., have also been used.

The selection criteria for a lactic acid bacteria to be used as 'probiotic' include the following ability to: (a) exert a beneficial effect on the host; (b) survive into a foodstuff at high cell counts, and remain viable throughout the shelf-life of the product; (c) withstand transit through the GI tract; (d) adhere to the intestinal epithelium cell lining and colonize the lumen of the tract; (e) produce antimicrobial substances towards pathogens; (f) stabilize the intestinal microflora and be associated with health benefits and (g) be nonpathogenic and nontoxic.

The roles of probiotics bacteria in dairy fermentations is to assist in: (a) the preservation of the milk by the production of lactic acid and probably antimicrobial compounds; (b) the production of flavor compounds and other metabolites that will provide a product with the organoleptic properties desired by the consumer; (c) to improve the nutritional value of food, as in, for example, the release of free amino acids or the synthesis of vitamins; and (d) the provision of special therapeutic or prophylactic properties as cancer and control of serum cholesterol levels.

Lowering serum cholesterol:

Animal studies

Many studies have used rats, mice, hamsters, guinea pigs and pigs as models due to their similarities digestive anatomy and physiology, nutrient requirements, bioavailability and absorption, and metabolic processes with humans in terms of cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of hepatic cholesterol enzymes.

Also several researchers have compared the effects of pasteurized milk, yogurt, and other fermented milks on cholesterol concentrations in animal models.

Gilliland *et al.* (1985) showed the cholesterol-reducing activity of Lactobacillus casei. In addition, this author (1975) reported that consumption of L. acidophilus RP32, which grew on bile and assimilated cholesterol from a laboratory medium, significantly inhibited increases in the serum cholesterol level of pigs fed on a high-cholesterol diet. In other study, Mahrous *et al* (2011) demonstrated that the cholesterol and bile acid levels in the serum of mice fed with yoghurt fermented by Lb. acidophilus P106 decreased significantly, while the cholesterol and bile acid content increased in mice feces. These effects may be due in part to the deconjugation of bile salts by strains of bacteria that produce the enzyme bile salt hydrolase (BSH). Moreover, Chiu et al (2006) observed cholesterol reduction and LDL levels in hamsters with high blood cholesterol levels which fed on milk fermented by L. paracasei subsp. paracasei NTU 101, L. plantarum NTU 102, and L. acidophilus BCRC 17010. In this report, HDL and TG contents also were reduced in the hamsters fed on diet containing lactic acid bacteria.

Sindhu and Khetarpaul conducted another study to estimate the effects of a probiotic fermented food on serum cholesterol levels in 20 young Swiss mice. The group was fed a food mixture containing probiotics and 1% cholesterol while the control group was fed with food containing 1% cholesterol but without probiotics for 42 days. They found that the feeding of L. casei NCDC-19 (109 CFU) and Saccharomyces boulardii (109 CFU) caused reduction in the total serum cholesterol (19%), while LDL cholesterol levels was reduced after the 42 day feeding trial by 37%.

In another study, Abd El-Gawad *et al* tested a randomized, placebo-controlled and parallel designed study to assess the efficiency of buffalo milk-yogurts which fortified with Bifidobacterium longum Bb-46 in exerting a cholesterol-lowering effect. In this research, forty-eight male albino fed for 35 days daily by hypercholesterolemia rats with average weight of 80-100 g, by 50 g of yogurt (contained 0.07% (w/v) *Bifidobacterium longum* Bb-46). The administration of *B. longum* Bb-46-fermented buffalo milk-yogurt significantly reduced concentration of total cholesterol (50.3%), LDL-cholesterol (56.3%) and triglycerides (51.2%) compared to the control ($P < 0.05$).

Human studies

Foods with lipid-rich have resulted in risks for heart diseases in recent years. For the first time, Mann and Spoerry (1974) discovered hypocholesterolemic effects arising from the diet of the Massai tribespeople in Africa, who ingested large intakes of milk fermented by Lactobacillus strains. Harrison and Peat showed that serum cholesterol levels decreased in newborn fed on fresh milk when the fecal titer of Lactobacillus acidophilus was raised. Hepner (1979) observed that reduction of serum cholesterol of people who used either non-pasteurized or pasteurized yoghurt is higher than who consumed 2% butterfat milk after 1 week. In this study serum triglycerids were unaffected by these diet. The cholesterol-reducing abilities of six strains of L. acidophilus were investigated and it was reported that in vivo cholesterol lowering ability was due to the assimilation of cholesterol by L. acidophilus cells or attachment of cholesterol to the surface of L. acidophilus cells. This bacteria being the natural inhabitant of intestine and possessing bile-salt hydrolase activity, can be exploited for the manufacture of acidophilus milk and its application as a means for reducing cholesterol level is recommended. Sarkar (2003) in his study reported that some factors affect the efficacy of acidophilus milk to lower serum cholesterol like the type of milk employed for product manufacture, age, sex, food habits and initial concentration of cholesterol of tested subjects. In other two studies investigated the cholesterol-lowering effect of *E. faecium*.

At first, 29 men received milk fermented with a human strain of *E. faecium* (108-1011 CFU/L) and 2 strains of *S. thermophilus* and 28 men received acidified milk as a control. Consumption of milk fermented with *E. faecium* decreased serum cholesterol after 6 week, whereas consumption of the acidified milk had no effect. Then they performed a second, larger study with 87 men and women with the same design. They report that LDL concentrations decreased throughout the test, with a significantly larger decrease in the Enterococcus group at weeks 4 and 12 than in the placebo-group ($P < 0.05$). Though, at the end of the study period and after the follow-up (week 24 and 30 respectively), LDL concentrations were no longer significantly different. The authors recommend that both chemically fermented milk and milk fermented with *E. faecium* lower serum cholesterol, while *E. faecium* has a more rapid effect. They conclude the effect of probiotics on serum cholesterol is inconclusive. In another study, Xiao et al. evaluated the effects of a low-fat yogurt containing 108 CFU/g of *B. longum* BL1 on lipid profiles of thirty-two subjects. Results from this research showed a significant ($P < 0.05$) decline in serum total cholesterol, LDL-cholesterol and triglycerides after 4-weeks while mainly significant decrease in serum total cholesterol was found among subjects with moderate hypercholesterolemia (serum total cholesterol > 240 mg/dl). The authors also observed increase in serum HDL-cholesterol when comparing to the control. The other research (2002) showed that the ingestion of 300 g youghurt fermented by traditional starter culture and supplemented with *L. acidophilus* and *B. longum* did not lower the total and LDL cholesterol in 29 healthy women. The effect of the HDL cholesterol may be due to the long-term consumption of yoghurt (21 weeks) and lead to improvement of the LDL/HDL ratio. Although many studies demonstrated cholesterol-lowering effects of probiotics in both animals and humans, some results are different, for instance, Hatakka *et al.* reported that the administration of *L. rhamnosus* LC705 (two capsules daily containing 1010 CFU/g per capsule) in thirty-eight men with mean cholesterol levels of 6.2 mmol/L after a 4-week treatment period, did not influence on total cholesterol, HDL cholesterol and triglyceride levels.

Bile: Bile is a yellow-green aqueous solution which major constituents include bile acids, cholesterol, phospholipids, and the pigment biliverdin. Bile functions as a biological detergent that emulsifies and solubilizes lipids, thereby playing an essential role in fat digestion. It is synthesized in the pericentral hepatocytes of the liver, stored and concentrated in the gallbladder interdigestively, and released into the duodenum where they are intimately associated with dietary lipids and various digestive products after food intake. Bile acids are excreted in the form of conjugate by the liver and conjugated bile acids are poorly absorbed by passive diffusion in the small and large intestines and mainly absorbed at the terminal ileum by the active transport mechanisms (Lack and Weiner, 1966), which is called ileum bile acid transporter (IBAT). After absorption, the mixture of bile salts is partly returned to the liver by the hepatic portal circulation in the process known as enterohepatic circulation (EHC). Roughly 600 to 800 ml of bile is produced every day. This is called ileum bile acid transporter (IBAT).

While deconjugated bile acids are less soluble and absorbed by the intestines, leading to their elimination in the feces. In animals all bile acids present in feces are in their free forms, while in man the bile acids are conjugated mainly to taurine or glycin. Therefore, deconjugation of bile salts could lead to a reduction in serum cholesterol in two ways, either by raising the demand for cholesterol for de novo synthesis of bile acids to replace those lost in feces or by reducing cholesterol solubility and thus absorption of cholesterol through the intestinal lumen.

Metabolism of bile acids in gastrointestinal microflora: Through bile acids flowing in large amounts in the digestive tract, the complex autochthonous gastrointestinal microflora have evolved the ability to transform the bile salts to a great size. The intestinal microflora can produce about 20 different bile acid metabolites from cholic acid and chenodeoxycholic acid. Recognized biotransformation by GI microorganisms includes deconjugation of the conjugated bile salts to release free bile acids and amino acid moiety, elimination of hydroxyl groups principally the 7 carbon hydroxyl group of the cholic acid moiety, oxidative and reductive reactions of the existing hydroxyl groups and epimerization of bile acids. Along with those reactions, bile salt deconjugation is the most biologically major reaction in that it performs a 'gatekeeping' function: hydrolysis of the conjugate bile acids is a prerequisite for any sterol transformation. The classes of microbial enzymes that catalyze hydrolysis of conjugated bile salts have been collectively named conjugated bile salt hydrolases, which is also called cholylglycine hydrolases.

Characteristic of BSH enzymes: BSHs have been purified and characterized from various microorganisms. They are oxygen insensitive, and have slightly acidic pH optima (usually between pH 5 and 6). Bile salt hydrolases (BSHs) are in the choloylglycine hydrolase family (EC 3.5.1.24) which hydrolyzes the amide bond and release the glycine or taurine moiety from the steroid core. The resulting acids are termed unconjugated or deconjugated bile acids. This enzyme differs in some characteristic like subunit size and composition, pH optimum kinetic properties, substrate specificity, gene organization, and regulation.

Mechanism of cholesterol-lowering

Gilliland and co-workers (1985) in an in vitro study found that certain Lactobacillus acidophilus strains could remove cholesterol from a growth medium only in the presence of bile and under anaerobic conditions. Because these conditions are expected to occur in the intestine, the authors concluded that this should enable the organisms to assimilate at least part of the cholesterol ingested in the diet, thus making it unavailable for absorption into the blood. However, the ability to grow in the presence of bile and to remove cholesterol from medium in vitro was found to vary considerably among *L. acidophilus* strains. It was suggested that an organism must be bile tolerant to manifest cholesterol uptake in the intestinal tract. Subsequent work, by Klaver and van der Meer (1993), studied the mechanism of the proposed assimilation of cholesterol by Lactobacillus acidophilus and *Bifidobacterium bifidum* in the presence of cholesterol and oxgall. They concluded that removal of cholesterol from the culture medium by L. acidophilus RP32 and other species was not due to bacterial uptake of cholesterol but rather could relate to co-precipitation with deconjugated bile salts in an acidic environment. Deconjugated bile acids are less soluble and less likely to be absorbed from the intestinal lumen than conjugated bile salts. In other research, Tahri *et al.* (1996) studied the hypothesis of the proposed assimilation or coprecipitation of cholesterol by *Bifidobacterium* species in medium containing oxgalls. They observed the existence of an intense binding between cell surface and cholesterol. They concluded that growing bifidobacteria cells are able to remove cholesterol both by precipitation and assimilation. Liong and Shah (2005) also found that bifidobacteria strains were able to assimilate cholesterol ranging from 4.17 to 27.14µg/Ml. in this study, heat-killed cells and resting-cells in phosphate buffer were also reduce cholesterol by binding, ranging from 1.11 to 3.35 mg/dry weight. In other study, they (2004) screened eleven strains of lactobacilli and analyzed bile salt deconjugation ability, bile salt hydrolase activity (BSH) and co-precipitation of cholesterol with deconjugated bile. *Lactobacillus acidophilus* strains had higher deconjugation ability than *L. casei* strains. In another study, Lactobacillus brevis, *Lactobacillus pentosus* and *Pedoicoccus acidilactici* and *Lactobacillus paracasei* which isolated from traditional Iranian yoghurt and sour buttermilk assimilated Cholesterol by both viable and dead cells in MRS broth containing 0.3% bile salt. All viable cells were able to lower the cholesterol to some extent, however the highest level of cholesterol removal was observed in Lactobacillus brevis which was able to reduce cholesterol in both forms of viable and dead cell. In other investigate, Seok, (1987) concluded that *L. sporogenes* lowers LDL cholesterol by eliminating it directly from inside the intestines before it can be absorbed into the blood stream. In another clinical study, *L. sporogenes* not only lowered total serum cholesterol and LDL cholesterol in humans, it also improved the ratio of "good" HDL cholesterol to total cholesterol. In addition, *P. acidipropionici* has been shown to reduce serum cholesterol levels in mice. In addition to other mechanism, the production of propionic acid may contribute to the lowering of serum cholesterol. Propionic acid bacteria, which are main propionate producers, may in that way contribute to lowering of serum cholesterol. In another study, observed that two possible mechanisms can underlie the ability of lactococci to remove cholesterol from media. One is adhesion of the cholesterol to the cell surface. It has been suggested that binding of cholesterol to the lactic acid bacterial cells may be a physical phenomenon and be related to the cell wall. Another possible mechanism is an assimilation of cholesterol by the cells. In addition, Parvez *et al*, (2005) showed that Growing cells of *Bifidobacterum bifidum* NRRL 1976 can reduce cholesterol due to both bacterial assimilation and precipitation of cholesterol.

In summarize, the cholesterol reduction produced by lactic acid bacteria can be explained by five mechanisms as follows: (1) fermentation products of lactic acid bacteria inhibit the activity of enzymes for cholesterol synthesis and thus reduce cholesterol production; (2) the bacteria facilitate the elimination of body cholesterol in feces; (3) the bacteria inhibit the absorption of cholesterol back into the body by binding with cholesterol; (4) the bacteria interfere with the recycling of bile salt (metabolic product of cholesterol) and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption; and, due to the assimilation of lactic acid bacteria, cholesterol in the host body is incorporated into the cell membrane or cell wall of bacteria to increase the resistance of bacterial cell membrane to environmental challenge; thus, the host cholesterol content is reduced.

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