



<b>Title</b>	<b>Probiotics and gut health: A special focus on liver diseases</b>
<b>Author(s)</b>	<b>Gratz, SW; Mykkanen, H; ElNezami, HS</b>
<b>Citation</b>	<b>World Journal Of Gastroenterology, 2010, v. 16 n. 4, p. 403-410</b>
<b>Issued Date</b>	<b>2010</b>
<b>URL</b>	<b><a href="http://hdl.handle.net/10722/123976">http://hdl.handle.net/10722/123976</a></b>
<b>Rights</b>	<b>Creative Commons: Attribution 3.0 Hong Kong License</b>

## Probiotics and gut health: A special focus on liver diseases

Silvia Wilson Gratz, Hannu Mykkanen, Hani S El-Nezami

Silvia Wilson Gratz, Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Aberdeen AB21 9SN, United Kingdom

Hannu Mykkanen, School of Public Health and Clinical Nutrition, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland

Hani S El-Nezami, Food and Health Research Centre, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland; School of Biological Sciences, University of Hong Kong, S5-13 Kadoorie Biological Sciences Building, Pokfulam, Hong Kong, China

Author contributions: Gratz SW, Mykkanen H and El-Nezami HS wrote the article.

Correspondence to: Hani S El-Nezami, Professor, School of Biological Sciences, University of Hong Kong, S5-13 Kadoorie Biological Sciences Building, Pokfulam, Hong Kong, China. [elnezami@hku.hk](mailto:elnezami@hku.hk)

Telephone: +852-22990835 Fax: +852-22990364

Received: October 23, 2009 Revised: December 4, 2009

Accepted: December 11, 2009

Published online: January 28, 2010

© 2010 Baishideng. All rights reserved.

**Key words:** Probiotics; Liver; Gut; Endotoxin; Barrier function; Ethanol; Bacterial overgrowth; Translocation

**Peer reviewers:** Xiaofa Qin, MD, PhD, Department of Surgery, UMDNJ-New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103, United States; Yasuji Arase, MD, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomon minato-ku, Tokyo 105-8470, Japan

Gratz SW, Mykkanen H, El-Nezami HS. Probiotics and gut health: A special focus on liver diseases. *World J Gastroenterol* 2010; 16(4): 403-410 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v16/i4/403.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i4.403>

### Abstract

Probiotic bacteria have well-established beneficial effects in the management of diarrhoeal diseases. Newer evidence suggests that probiotics have the potential to reduce the risk of developing inflammatory bowel diseases and intestinal bacterial overgrowth after gut surgery. In liver health, the main benefits of probiotics might occur through preventing the production and/or uptake of lipopolysaccharides in the gut, and therefore reducing levels of low-grade inflammation. Specific immune stimulation by probiotics through processes involving dendritic cells might also be beneficial to the host immunological status and help prevent pathogen translocation. Hepatic fat metabolism also seems to be influenced by the presence of commensal bacteria, and potentially by probiotics; although the mechanisms by which probiotic might act on the liver are still unclear. However, this might be of major importance in the future because low-grade inflammation, hepatic fat infiltration, and hepatitis might become more prevalent as a result of high fat intake and the increased prevalence of obesity.

### INTRODUCTION

Probiotics have been well defined and long used in human and animal health and nutrition. Many of the probiotic strains used today have been isolated from the human gut flora, and it is therefore more a reintroduction of organisms rather than a novel concept. The beneficial effects of probiotics and, especially, the clinical use of probiotics in the management of specific diarrhoeal diseases, including Rotavirus diarrhoea, Traveller's diarrhoea and others are well accepted<sup>[1]</sup>. These effects are mainly based on colonisation resistance or the influence of probiotics on microflora balance. When discussing probiotics, it must be remembered that the intestinal microflora, resident in the large intestine, will always outnumber the probiotics that can be administered. Furthermore, probiotic processes will always be confounded by the diversity of the human microbiota and its variability in the face of varied human diets and genetic backgrounds<sup>[2]</sup>.

Within the human gut, we have to separate processes occurring in the distal small intestine from those happening in the colon. The small intestine harbours relatively low numbers of resident intestinal bacteria,

but at the same time contains the major part of the gut associated lymphoid tissue (GALT), which samples intestinal microbes<sup>[3]</sup>. Hence modulation of systemic immune and allergic phenomena might be primarily mediated by the GALT of the small bowel. Supplementing qualitatively and quantitatively optimised microbes to this part of the gut might stimulate Treg cell development and consequent immunomodulation<sup>[1]</sup>. Within the large intestine, bacterial intervention might cause modulation of the microbial fermentation activity and direct action on the colonic epithelium to alter (suppress) innate immunity. These processes might explain the impact of probiotics on inflammatory and functional bowel disorders.

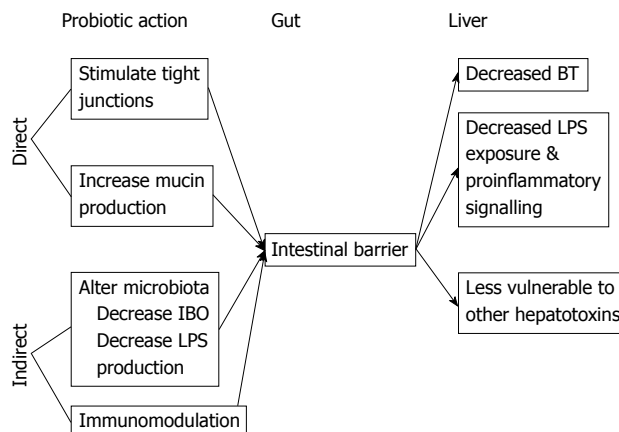
The research field of probiotics is very heavily reviewed, using different clinical and microbiological angles to elucidate the topic. The aim of this review is to summarise the most recent trends in probiotic research, focusing on the clinical use of probiotics and their effects on the healthy and diseased gut and liver.

The direct and indirect actions of probiotics on intestinal cells and their consequences on liver (summarised in Figure 1) will be discussed in this review.

## GUT HEALTH

### Probiotics and intestinal barrier function

The intestinal barrier is seen as the first line of defence against pathogens and food allergens entering the intestinal tract, and probiotics have been intensely studied for their involvement in maintaining this barrier. In colonic epithelia, probiotics are suggested to stimulate mucin production, and therefore enhance the self protecting properties of the intestinal epithelium<sup>[4]</sup>. Furthermore, tight junctional proteins, important for the physical tightness of the epithelial cell layer, are found to be enhanced by probiotics<sup>[5,6]</sup> and the disruption of tight junctions by pathogens can be counteracted<sup>[7]</sup>. Besides these effects on the physical barrier function, evidence is mounting that both commensal microflora and specific probiotic supplementations specifically enhance the immunological barrier function of the small intestinal mucosa<sup>[8]</sup>. For this cross talk between bacteria and the immune system, bacteria derived products including metabolites, cell wall components and DNA, can be sensed by enterocytes and immune competent cells<sup>[9]</sup>. Commensals, unlike pathogens, are efficiently killed by intestinal macrophages, therefore avoiding an inflammatory response in the mucosa<sup>[10]</sup>. At the same time, dendritic cells can sample commensals, incorporate them, and transport them to mesenteric lymph nodes. Here, commensal-loaded dendritic cells induce a local immune response with the activation of specific B-cells to produce secretory IgA against these commensals<sup>[8,10]</sup>. This appears to be a paradox, but this specific and local immune stimulation by commensals and probiotics can actually be considered non-inflammatory in the mucosal environment and the host systemics<sup>[8]</sup>. Therefore GALT,



**Figure 1 Potential mechanisms of action by which probiotics can promote GI health and the consequences for the liver.** Probiotics and surface-layer proteins competitively exclude microbial pathogens from mucosal surfaces. Tight junction proteins, such as zona occludins-1 and claudin1, remain intact and thereby prevent both uptake of intact macromolecules and translocation of viable organisms (BT) to mesenteric lymph nodes, and ultimately to the liver. Through a cascade of signalling events, probiotics enhance production and secretion of anti-inflammatory cytokines, including interleukin-10 and transforming growth factor- $\beta$ , by a subset of immune cells, referred to as T regulatory cells. Innate immune responses to probiotics include increased mucin and trefoil factor production by goblet cells and enhanced production of antibacterial defensins by Paneth cells and intestinal epithelia. Probiotics might alter the intestinal microbiota and hence limit intestinal bacteria overgrowth (IBO) and the production of lipopolysaccharides (LPS).

and specifically the mesenteric lymph nodes, can be considered another layer of intestinal barrier function<sup>[3,10]</sup>.

### Probiotics and inflammatory bowel diseases (IBD)

The pathogenesis of IBD [ulcerative colitis (UC) and Crohn's disease] remains unknown, but the intestinal microflora appears to play an important role. Changes in microflora composition have been observed in UC patients, with increased pro-inflammatory bacteria, including Enterobacteriaceae, increased *Bacteroides fragilis* within the mucosal microflora<sup>[11]</sup>, and decreased protective bacteria, including lactobacilli and bifidobacteria<sup>[12]</sup>. Probiotic treatment has the potential to decrease the severity of symptoms in IBD *via* interaction with gut epithelium<sup>[13]</sup>. Proposed mechanisms include changes in short chain fatty acids (SCFA) production patterns, reduction in pro-inflammatory cytokine secretion, improving Th1/Th2 ratios, and eliminating pathogens. For example, the production of reuterin has been shown *in vitro* to reduce growth of pathogens, including *Escherichia coli* (*E. coli*), *Salmonella enterica*, *Shigella sonnei*, and *Vibrio cholera*. Adhesion of probiotics to enterocytes and enhancement of barrier function (secretion of B-defensins and mucus, TJ proteins) have also been shown for specific strains *in vitro*. However, it is unclear whether this strengthening of the intestinal barrier function would also occur in the large intestine, the area most affected by intestinal disease. Some evidence from a mouse model of colitis [interleukin (IL)-10 deficient mice] suggests that probiotic lactobacilli and the VSL#3 mix reduced

bacterial translocation (BT) and intestinal permeability in the colon<sup>[14,15]</sup>. Mechanistic effects can also be observed with non-viable probiotics and culture supernatants *in vitro* and in animals, which might be a safer therapeutic for patients with impaired intestinal barrier and increased risk of sepsis. Bacteria-free culture supernatant from *Lactobacillus plantarum* was shown to inhibit inflammatory pathways important for intestinal inflammation, such as nuclear factor (NF)- $\kappa$ B binding activity and protease activity, in a young adult mouse colon cell line and in macrophages<sup>[16]</sup>. This might provide a novel and safe strategy for treatment of IBD, if results can be repeated *in vivo*.

### Probiotics and irritable bowel syndrome (IBS)

IBS includes a range of symptoms, such as abdominal pain, altered bowel habits, bloating and flatulence in the absence of structural abnormalities in the intestine. As no curative treatment is available for IBS, therapy is palliative and supportive, targeting special symptoms, and is notoriously unsatisfactory. Studies have observed alterations in intestinal microflora in patients and increased symptoms following enteric infections, therefore probiotics might be a useful tool to improve symptoms. A meta analysis of probiotic treatment and IBS<sup>[17]</sup> included 20 trials with 23 probiotic treatments. This study showed that probiotics were associated with improved global IBS symptoms [risk ratio (RR) = 0.77] and with decreased abdominal pain (RR = 0.78). Due to the large variety in probiotic strains used, no analysis on strain type was possible. Probiotics used included *B. infantis*, lactobacilli (*L. acidophilus*, *L. plantarum*, *L. reuteri*, *L. rhamosus*), *Saccharomyces boulardii*, *Streptococcus faecium*, VSL#3 (mix of eight), and other mixes. In a recent systematic review, Brenner *et al.*<sup>[18]</sup> claimed that 16 randomized controlled human trials met their inclusion criteria, of which 11 had suboptimal study design. They concluded that only one study using a specific strain of *B. infantis* 35624 efficiently improved IBS symptoms. Probiotics might offer a treatment possibility for IBS symptoms, but more controlled studies are needed to identify the ideal strain, dose, and duration of treatment.

### Probiotics after gut surgery

Morbidly obese patients can undergo Roux-en Y gastric bypass surgery (one type of bariatric surgery) for effective and enduring weight loss. This procedure uses restriction in stomach size and intestinal malabsorption as measures for achieving substantial weight loss. Problems that occur postoperatively are alteration of microflora with bacterial overgrowth (BO) in the blind sac of the intestine, intestinal pain, and possibly impaired vitamin B<sub>12</sub> status (due to lack of intrinsic factor production from the stomach). This study<sup>[19]</sup> used probiotic treatment (6 mo, commercial product of unspecified lactobacilli) in 44 post operational patients. BO (measured with hydrogen breath test) improved only after 6 mo, but not earlier. Vitamin B<sub>12</sub> status improved, gastrointestinal

symptoms remained unchanged, but post operational weight loss was significantly increased in the probiotic group. The authors speculated that increased weight loss might be due to changes in microflora towards extracting fewer calories from the diet, although this was not studied.

Probiotics/symbiotics have also been used to prevent postoperative infections in patients undergoing abdominal surgery (biliary cancer, liver transplantation, and pancreaticoduodenectomy). Major infections were pneumonia, urinary tract infection, wound infection, intra-abdominal abscess, and cholangitis. A recent meta analysis<sup>[20]</sup> found that probiotics reduced overall infections [odds ratio (OR) = 0.26], length of antibiotic treatment need (OR = -4.01), reduced length of postoperative hospital stays (OR = -2.7), but did not change overall mortality (OR = 0.98). Overall the use of probiotics is very promising, although data are very variable (type of surgery, type of infection, and type of probiotic treatment). However, bacteremia might be a potential hazard in these vulnerable patients. The appropriate therapeutic route, length of therapy, time of administration, dosage, and kind of probiotic remain controversial and no uniform preventative strategy can be suggested on based of the current literature.

## LIVER HEALTH

There is a longstanding practice of using lactulose in the treatment of hepatic encephalopathy, which suggests involvement of gut microflora in the management of chronic liver disease. Loguercio *et al.*<sup>[21]</sup> use the phrase "gut liver axis" and suggest that the microflora might affect the liver and be cofactor in aetiology of chronic liver damage. This could happen *via* modulating chronic damage by ethanol or by contributing to complications such as encephalopathy (production of ammonia, ethanol, acetaldehyde, phenols, endotoxin, and benzodiazepines)<sup>[21]</sup>. Probiotic actions most relevant to liver disease are modification of intestinal barrier function and prevention of BT. Gram-negative BO, increased permeability, and impaired immunity all contribute to increased BT, and there is a strong correlation between the rate of BT and the severity of cirrhosis. Probiotics might alter gut flora towards protective organisms and increase barrier function<sup>[22]</sup>.

### Probiotics and non alcoholic fatty liver disease (NAFLD)

NAFLD is the most common form of liver disease in the US; its incidence is rising together with rising problems of obesity and Type II diabetes. NAFLD includes a spectrum of pathologies. Steatosis (fatty liver), is clinically asymptomatic, but might predispose the liver to other insults, such as lipopolysaccharides (LPS) or hepatotoxins, that might lead to cirrhosis. Non-alcoholic steatohepatitis (NASH) is an intermediate state where lobular inflammation occurs. Cirrhosis is the most severe form, responsible for most liver specific morbidity and mortality<sup>[23]</sup>.

Histopathological changes are very similar to alcoholic liver disease, and there might be a common pathway of development. Data suggest a “multi hit” hypothesis, where initial hits such as obesity and sub-clinical insulin resistance might promote the development of steatosis<sup>[23]</sup>. This enhances the fatty liver’s vulnerability to subsequent insult (e.g. ethanol and LPS) that increase the production of pro-inflammatory cytokines [e.g. tumor necrosis factor (TNF)- $\alpha$ ]. This aggravates insulin resistance and leads to oxidative stress (increased production of reactive oxygen by hepatocytes and liver macrophages) and organelle dysfunctions, which kill hepatocytes and promote accumulation of inflammatory cells in the liver and the development of NASH<sup>[24]</sup>. Following years of chronic inflammation this might develop into fibrogenic response and cirrhosis.

The contribution of microflora in the development of NAFLD is mainly based on increased hepatic oxidative stress by increased production of ethanol and LPS in the intestinal lumen, and subsequent release of inflammatory cytokines in intestinal epithelia and liver macrophages. Both processes then lead to injury of the intestinal epithelium and disrupted intestinal barrier function, which in turn increases hepatic exposure to intestine-derived toxins. This hypothesis is further supported by evidence that intestinal BO exacerbates fatty liver disease in rodents and humans, and that obese patients with NASH have increased prevalence of BO. Furthermore, obese subjects are known to have decreased intestinal motility and are therefore more prone to BO. A recent clinical trial confirms that patients with NASH have increased intestinal permeability and small intestinal BO<sup>[25]</sup>.

From this data, an obvious way to control the development of NAFLD seems to be a manipulation of the gut microflora, mainly by the reduction of BO. This can be achieved by antibiotic treatment, which has been used successfully, but is controversial due to its unspecific impairment of all microflora and its severe side effects<sup>[23]</sup>. Probiotic therapy, on the other hand, has been suggested to counteract the development of NAFLD on various different levels. Competitive inhibition of pathogens by probiotics might alter their inflammatory effects in intestinal BO, which is associated with NAFLD. Furthermore, improved intestinal epithelium function and decreased BT and endotoxemia following probiotic treatment have been observed in experimental animals and humans<sup>[26]</sup>. In a series of feeding studies in mice, Cani *et al.*<sup>[27]</sup> claimed that high-fat feeding changes the intestinal microflora composition (less bifidobacteria), which led to increased LPS levels in plasma, pro-inflammatory cytokines and increased intestinal permeability. All these effects could be counteracted by prebiotic treatment and increasing bifidobacteria species (summarized in<sup>[27]</sup>). This would indicate a direct effect of intestinal bacteria on low-grade inflammation, insulin insensitivity, and fat deposition in the liver.

Other research suggests a direct decrease in pro-inflammatory cytokines e.g. TNF- $\alpha$  *via* downregulation of

NF- $\kappa$ B activity by probiotic treatments<sup>[14,28-30]</sup>. In a study in ob/ob mice, probiotics (VSL#3) and TNF- $\alpha$  antibodies were used to treat NAFLD. Both treatments improved liver function, reduced hepatic fatty acid content, and both interfered with NF- $\kappa$ B signalling and reduced hepatic fatty acid  $\beta$ -oxidation close to levels in lean mice. The authors suggest that this effect might result from improved hepatic insulin resistance<sup>[28]</sup>. Another study, using the same probiotic in normal mice, measured hepatic natural killer T (NKT)-cell depletion in high-fat fed animals<sup>[31]</sup>. NKT are unconventional T cells that express both T cell and Killer cell receptors. They regulate hepatic inflammatory process by balancing production of pro- and anti-inflammatory cytokines. Alterations of NKT function might lead to overproduction of TNF- $\alpha$ , causing inflammation in insulin resistance. High-fat diet induced the depletion of NKT from the liver, leading to insulin resistance and steatosis. Probiotics significantly improved all these symptoms and the effect resulted from TNF- $\alpha$  signalling and led to improved insulin signalling<sup>[31]</sup>. There is some suggestive evidence that probiotics might have efficacy in NAFLD in humans, but more controlled trials are needed<sup>[30]</sup>.

#### **Probiotics and alcoholic liver disease**

In alcoholic liver disease, bowel liver interactions are well described, and relationships include increased gut permeability, endotoxemia, and TNF- $\alpha$  production<sup>[32]</sup>. In rats, *Lactobacillus* GG has been shown to reduce alcohol induced gut leakiness and steatohepatitis<sup>[33]</sup>. The same group also found that the mucosa associated microflora were altered in rats on a high alcohol feed, and this dysbiosis could be counteracted by *Lactobacillus* GG or oat supplementation<sup>[34]</sup>. Furthermore, alcoholics have altered microflora with decreased numbers of bifidobacteria and lactobacilli. A recent pilot study<sup>[32]</sup> compared control subjects with alcoholics in a clinic, either on standard treatment for alcoholic disease (Vitamin B<sub>1</sub> & B<sub>6</sub> supplements and diazepam,  $n = 34$ ) or on standard treatment + probiotics ( $0.9 \times 10^8$  CFU *B. bifidum* and  $0.9 \times 10^9$  CFU *L. plantarum*,  $n = 32$ ) for five consecutive days. No placebo was included in the control group. They found that lactobacilli, bifidobacteria, and enterococci were reduced in alcoholics and the numbers were restored in the probiotic treatment group. *E. coli* levels were not altered. Liver function parameters [alanine aminotransferase (ALT) and aspartate aminotransferase] were significantly improved by probiotics compared to standard treatment, but remained below levels found in healthy controls. Other liver enzymes (GGT and LDH) were not significantly altered. In the subgroup of alcoholics with well-defined hepatitis, standard treatment only altered total bilirubin, but probiotics improved all the liver parameters mentioned above. The authors concluded that bifidobacteria to play an important role in steatohepatitis of alcoholic and non alcoholic causes, including obesity<sup>[35]</sup>. In a double blind placebo

controlled (sucrose capsule) intervention Lata *et al*<sup>[36]</sup> studied the effect of probiotic *E. coli* Nissle in cirrhotic patients ( $n = 39$ , 34 with alcoholic cirrhosis). They found an improvement of intestinal colonization in faeces, and a restoration of “physiological microflora” in faeces (more patients with normal microflora containing lactobacilli and bifidobacteria, less patients with potentially pathogenic bacteria). They also showed trends towards a reduced endotoxin level in blood ( $P = 0.07$ ) and a reduced Child-Pugh score ( $P = 0.07$ ), which is a measure for severity of overall liver disease. Improved liver function by probiotics has already been published<sup>[5]</sup>, but the mechanism remains unclear. They assume that microflora reduce the toxic load of liver, e.g. reduced endotoxin, which stimulates pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, 6)<sup>[22]</sup>.

### Probiotics to cure/alleviate cirrhosis?

In cirrhosis there are many conditions that alter microflora and the function of the intestinal epithelium, as recently reviewed in detail<sup>[37]</sup>. BT is caused by BO, increased permeability, and altered host defence. BT and microflora imbalance are strongly correlated with the severity of cirrhosis<sup>[38]</sup>. In a rat model of acute liver injury (using either D-galactosamine or endotoxin), probiotic lactobacilli and bifidobacteria attenuated liver injury (ALT and bilirubin), reduced BT, and normalized hepatic TNF- $\alpha$  and glutathione levels compared to liver injury controls<sup>[39,40]</sup>.

Lactobacilli might counteract BT by (1) promoting growth of anaerobes and Gram-positive bacteria while inhibiting gram-negative bacteria, or (2) increasing SCFA while decreasing pH, inducing growth factors and proliferation of microflora, and inhibiting adherence and invasion of pathogens. Patients with liver cirrhosis have imbalanced intestinal microflora with increased aerobes (enterobacter and enterococci) and anaerobes (clostridia), and decreased bifidobacteria counts in stool. In a probiotic intervention trial in cirrhotic patients<sup>[38]</sup> (mainly caused by hepatitis virus B and C; HBV and HCV) patients received two different probiotic capsules [bifidobacteria + *L. acidophilus* + *Enterococcus* or *Bacillus subtilis* (*B. subtilis*) + *Enterococcus faecium* (*E. faecium*)], two capsules per day for 14 d. In both probiotic groups, bifidobacteria counts increased with treatment, while faecal pH and ammonia levels in faeces and blood decreased. Additionally, *B. subtilis* + *E. faecium* decreased clostridia counts and endotoxin levels in the blood of cirrhotic patients.

Flora imbalance in cirrhotics might be caused by decreased gut motility, diminished excretion of secretory IgA, lysozyme, mucus, acids, increased pH, shortage of bile acids, and excessive alcohol intake. Elevated blood ammonia is a crucial factor in hepatic encephalopathy aetiology.

Loguercio *et al*<sup>[21]</sup> conducted a pilot study in patients suffering from hepatitis of various causes (HCV, alcoholism, and NASH). The patients received a probiotic

mix (*Lactobacillus acidophilus*, *L. bifidus*, *L. rhamnosus*, *L. plantarum*, *L. salivarius*, *L. bulgaricus*, *L. lactis*, *L. casei*, *L. breve* + fructo-oligosaccharides + vitamins). Interestingly, the authors reported no effect of probiotics in HCV patients. In NASH patients some liver function parameters (ALT and  $\gamma$ -glutamyltransferase) improved, TNF- $\alpha$  decreased and plasma malondialdehyde decreased in some patients with probiotic treatment. The strongest effect of probiotics was seen in patients with alcoholic liver cirrhosis, where all parameters of liver function improved, as did TNF- $\alpha$  and malondialdehyde.

From these studies in humans, it appears that the microflora is an important cofactor in the aetiology of chronic liver disease, and that probiotics might have a therapeutic role.

### Probiotics to bind toxins and carcinogens

Some experimental evidence suggests that probiotics could be used to bind and immobilise toxic compounds within the gut lumen. Through this process, the negative effects of dietary toxins could be reduced and gut and liver health improved. *In vitro*, *L. rhamnosus* GG is able to bind mycotoxins known to interfere with intestinal mucosal barrier. In Caco-2 cells, the negative effects of mycotoxins on cell differentiation and intestinal integrity can be attenuated with *L. rhamnosus* GG<sup>[41,42]</sup>. In rats, genotoxic effects of food carcinogens, such as heterocyclic amines, on the colonocytes and hepatocytes were counteracted by the use of different probiotics<sup>[43,44]</sup>. Probiotics have also been shown to attenuate hepatotoxic effects of aflatoxin, a well known liver carcinogen, in rats<sup>[45]</sup> and to reduce biomarkers of liver cancer risk in a human intervention trial<sup>[46]</sup>.

### Safety considerations of probiotics

Generally recognized as safe (GRAS) status is defined by the Food and Drug Administration for food adjuncts that might not meet the usual requirements for safety assessment but have been used extensively without demonstrable harm. Probiotics are claimed to be GRAS as they comprise organisms identical to those in human gut and vaginal flora, although strain dependence needs to be considered and GRAS should only be granted to one specific probiotic preparation used in one specific food product<sup>[47]</sup>. Previous probiotic studies stress the safety of probiotic preparations and their ability to reduce BT of pathogenic bacteria to host organs and tissues. Probiotic BT from the intestine is difficult to induce in healthy animals, and therefore hard to study. From animal studies, NOAEL (no observed adverse effect levels) can be determined and ADI (acceptable daily intake) extrapolated for humans. These calculations suggest that up to  $10^{14}$  cfu/d of lactobacilli and bifidobacteria, a dose way beyond the usual intake of  $10^9$ - $10^{11}$  cfu/d, are acceptable for human consumption. In healthy humans, probiotic BT occurs occasionally. but detrimental effects are rare. Salminen *et al*<sup>[48]</sup> assessed the frequency of lactobacillus bacteraemia in the Finnish population

following the increased consumption of probiotic products in the years 1995-2000 and found no trends towards increased lactobacillus bacteraemia over this period. A recent meta analysis summarised the safety of probiotics in pregnancy and concluded no effect of lactobacillus and bifidobacterium species on incidence of caesarean section, birth weight, or gestational age<sup>[49]</sup>. In immunocompromised individuals however, this might be different. Cannon *et al.*<sup>[50]</sup> summarised over 200 clinical cases of lactobacillus infections and found association with endocarditis and bacteraemia. *L. casei* and *rhamnosus* were most common, and the overall mortality rate was nearly 30%. They also reported that the main underlying conditions were cancer, diabetes, antibiotic therapy, organ transplantation, and abscesses. Salminen *et al.*<sup>[51]</sup> investigated the severity and outcome of lactobacillus bacteraemia in 89 patients, and report mortality of 26% 1 mo after illness onset, but only in patients with severe underlying comorbidities.

### Future of probiotic research

Most recent developments in probiotic and prebiotic research use a new systems biology approach to assess the complex relationship between the microflora, probiotic modulations, and the impact on host metabolism in multiple compartments. A major finding is the impact of microflora modulation on host energy metabolism, especially lipid metabolism in the liver where marked decreases in plasma lipoproteins and hepatic glutamine and glycogen levels are observed<sup>[52]</sup>. This group uses a germ free mouse model colonized with human baby flora, to study the effects of a probiotic intervention (*L. paracasei* or *L. rhamnosus*) on gut flora composition, SCFA in caecal content, plasma, urine, faecal and liver metabolomics, and bile acids in ileal flushes.

The integration of multicompartiment metabolic data using hierarchical principal component analysis showed that probiotic lactobacilli induce changes in hepatic influx and efflux of fatty acids, increased enterohepatic recycling of bile acids and dietary fats, lowered plasma LP, and stimulated glycolysis. Probiotic intervention also changed the proteolytic activity and bacterial metabolism of AA and SCFA in the gut<sup>[53]</sup>. In a mouse model of high-fat feeding and NAFLD, an association was shown between the metabolism of choline by microbiota and the host. The author suggested a contribution of microflora to the development of the NAFLD phenotype<sup>[54]</sup>. This complex analytical and statistical methodology allows investigation of the impact of microflora and probiotics on various body compartments simultaneously and might, in the future, lead to a better understanding of the influence of probiotics on the host. Similarly, the complex metabolic relationships between the microflora and the host have also been studied in human cohorts and this approach might be used to gain further understanding of the relation between changes in the microflora (dysbiosis) and disease<sup>[55]</sup>.

## CONCLUSION

It appears that specific clinical applications of probiotics are safe, effective, and can clearly be recommended. However, the importance of probiotic food items in the “maintenance of health” in healthy individuals as marketed by food industries remains questionable. To date, no generalisation can be made from health effects of one probiotic strain to another one and this remains a serious problem within the probiotic research field and its applications. Multi-dimensional research approaches, studying the microflora composition, its metabolic profile, and the impact on host metabolism appear a promising way forward to further describe and explore these complex relationships within the microflora-host “superorganism”.

## REFERENCES

- 1 **Ouweland AC.** Antiallergic effects of probiotics. *J Nutr* 2007; **137**: 794S-797S
- 2 **Neish AS.** Microbes in gastrointestinal health and disease. *Gastroenterology* 2009; **136**: 65-80
- 3 **Cummings JH,** Antoine JM, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, Pannemans D, Shortt C, Tuijelaars S, Watzl B. PASSCLAIM—gut health and immunity. *Eur J Nutr* 2004; **43** Suppl 2: II118-II173
- 4 **Caballero-Franco C,** Keller K, De Simone C, Chadee K. The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G315-G322
- 5 **Otte JM,** Podolsky DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G613-G626
- 6 **Commans DM,** Shortt CT, Silvi S, Cresci A, Hughes RM, Rowland IR. Effects of fermentation products of pro- and prebiotics on trans-epithelial electrical resistance in an in vitro model of the colon. *Nutr Cancer* 2005; **51**: 102-109
- 7 **Johnson-Henry KC,** Donato KA, Shen-Tu G, Gordanpour M, Sherman PM. Lactobacillus rhamnosus strain GG prevents enterohemorrhagic Escherichia coli O157:H7-induced changes in epithelial barrier function. *Infect Immun* 2008; **76**: 1340-1348
- 8 **Isolauri E,** Salminen S. Probiotics, gut inflammation and barrier function. *Gastroenterol Clin North Am* 2005; **34**: 437-450, viii
- 9 **Corthésy B,** Gaskins HR, Mercenier A. Cross-talk between probiotic bacteria and the host immune system. *J Nutr* 2007; **137**: 781S-790S
- 10 **Macpherson AJ,** Uhr T. Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. *Science* 2004; **303**: 1662-1665
- 11 **Wang M,** Molin G, Ahrné S, Adawi D, Jeppsson B. High proportions of proinflammatory bacteria on the colonic mucosa in a young patient with ulcerative colitis as revealed by cloning and sequencing of 16S rRNA genes. *Dig Dis Sci* 2007; **52**: 620-627
- 12 **Cummings JH,** Macfarlane GT, Macfarlane S. Intestinal bacteria and ulcerative colitis. *Curr Issues Intest Microbiol* 2003; **4**: 9-20
- 13 **Prisciandaro L,** Geier M, Butler R, Cummins A, Howarth G. Probiotics and their derivatives as treatments for inflammatory bowel disease. *Inflamm Bowel Dis* 2009; **15**: 1906-1914

- 14 **Madsen K**, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; **121**: 580-591
- 15 **Madsen KL**, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116**: 1107-1114
- 16 **Petrof EO**, Claud EC, Sun J, Abramova T, Guo Y, Waypa TS, He SM, Nakagawa Y, Chang EB. Bacteria-free solution derived from Lactobacillus plantarum inhibits multiple NF-kappaB pathways and inhibits proteasome function. *Inflamm Bowel Dis* 2009; **15**: 1537-1547
- 17 **McFarland LV**, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World J Gastroenterol* 2008; **14**: 2650-2661
- 18 **Brenner DM**, Moeller MJ, Chey WD, Schoenfeld PS. The utility of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Am J Gastroenterol* 2009; **104**: 1033-1049; quiz 1050
- 19 **Woodard GA**, Encarnacion B, Downey JR, Peraza J, Chong K, Hernandez-Boussard T, Morton JM. Probiotics improve outcomes after Roux-en-Y gastric bypass surgery: a prospective randomized trial. *J Gastrointest Surg* 2009; **13**: 1198-1204
- 20 **Pitsouni E**, Alexiou V, Saridakis V, Peppas G, Falagas ME. Does the use of probiotics/synbiotics prevent postoperative infections in patients undergoing abdominal surgery? A meta-analysis of randomized controlled trials. *Eur J Clin Pharmacol* 2009; **65**: 561-570
- 21 **Loguercio C**, De Simone T, Federico A, Terracciano F, Tuccillo C, Di Chicco M, Carteni M. Gut-liver axis: a new point of attack to treat chronic liver damage? *Am J Gastroenterol* 2002; **97**: 2144-2146
- 22 **Sheth AA**, Garcia-Tsao G. Probiotics and liver disease. *J Clin Gastroenterol* 2008; **42** Suppl 2: S80-S84
- 23 **Solga SF**, Diehl AM. Non-alcoholic fatty liver disease: lumen-liver interactions and possible role for probiotics. *J Hepatol* 2003; **38**: 681-687
- 24 **Medina J**, Fernández-Salazar LI, García-Buey L, Moreno-Otero R. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. *Diabetes Care* 2004; **27**: 2057-2066
- 25 **Miele L**, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, Mascianà R, Forgione A, Gabrieli ML, Perotti G, Vecchio FM, Rapaccini G, Gasbarrini G, Day CP, Grieco A. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* 2009; **49**: 1877-1887
- 26 **Eizaguirre I**, Urkia NG, Ansio AB, Zubillaga I, Zubillaga P, Vidales C, Garcia-Arenzana JM, Aldazabal P. Probiotic supplementation reduces the risk of bacterial translocation in experimental short bowel syndrome. *J Pediatr Surg* 2002; **37**: 699-702
- 27 **Cani PD**, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des* 2009; **15**: 1546-1558
- 28 **Li Z**, Yang S, Lin H, Huang J, Watkins PA, Moser AB, Desimone C, Song XY, Diehl AM. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* 2003; **37**: 343-350
- 29 **Esposito E**, Iacono A, Bianco G, Autore G, Cuzzocrea S, Vajro P, Canani RB, Calignano A, Raso GM, Meli R. Probiotics reduce the inflammatory response induced by a high-fat diet in the liver of young rats. *J Nutr* 2009; **139**: 905-911
- 30 **Lirussi F**, Mastropasqua E, Orando S, Orlando R. Probiotics for non-alcoholic fatty liver disease and/or steatohepatitis. *Cochrane Database Syst Rev* 2007; CD005165
- 31 **Ma X**, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. *J Hepatol* 2008; **49**: 821-830
- 32 **Kirpich IA**, Solovieva NV, Leikhter SN, Shidakova NA, Lebedeva OV, Sidorov PI, Bazhukova TA, Soloviev AG, Barve SS, McClain CJ, Cave M. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. *Alcohol* 2008; **42**: 675-682
- 33 **Forsyth CB**, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. Lactobacillus GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol* 2009; **43**: 163-172
- 34 **Mutflu E**, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcohol Clin Exp Res* 2009; **33**: 1836-1846
- 35 **Cani PD**, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007; **50**: 2374-2383
- 36 **Lata J**, Novotný I, Příbramská V, Juránková J, Fric P, Kroupa R, Stibůrek O. The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. *Eur J Gastroenterol Hepatol* 2007; **19**: 1111-1113
- 37 **Guerrero Hernández I**, Torre Delgadillo A, Vargas Vorackova F, Uribe M. Intestinal flora, probiotics, and cirrhosis. *Ann Hepatol* 2008; **7**: 120-124
- 38 **Zhao HY**, Wang HJ, Lu Z, Xu SZ. Intestinal microflora in patients with liver cirrhosis. *Chin J Dig Dis* 2004; **5**: 64-67
- 39 **Adawi D**, Ahrné S, Molin G. Effects of different probiotic strains of Lactobacillus and Bifidobacterium on bacterial translocation and liver injury in an acute liver injury model. *Int J Food Microbiol* 2001; **70**: 213-220
- 40 **Osman N**, Adawi D, Ahrné S, Jeppsson B, Molin G. Endotoxin- and D-galactosamine-induced liver injury improved by the administration of Lactobacillus, Bifidobacterium and blueberry. *Dig Liver Dis* 2007; **39**: 849-856
- 41 **Gratz S**, Wu QK, El-Nezami H, Juvonen RO, Mykkänen H, Turner PC. Lactobacillus rhamnosus strain GG reduces aflatoxin B1 transport, metabolism, and toxicity in Caco-2 cells. *Appl Environ Microbiol* 2007; **73**: 3958-3964
- 42 **Turner PC**, Wu QK, Piekola S, Gratz S, Mykkänen H, El-Nezami H. Lactobacillus rhamnosus strain GG restores alkaline phosphatase activity in differentiating Caco-2 cells dosed with the potent mycotoxin deoxynivalenol. *Food Chem Toxicol* 2008; **46**: 2118-2123
- 43 **Zsivkovits M**, Fekadu K, Sontag G, Nabinger U, Huber WW, Kundi M, Chakraborty A, Foissy H, Knasmüller S. Prevention of heterocyclic amine-induced DNA damage in colon and liver of rats by different lactobacillus strains. *Carcinogenesis* 2003; **24**: 1913-1918
- 44 **Terahara M**, Meguro S, Kaneko T. Effects of lactic acid bacteria on binding and absorption of mutagenic heterocyclic amines. *Biosci Biotechnol Biochem* 1998; **62**: 197-200
- 45 **Gratz S**, Täubel M, Juvonen RO, Viluksela M, Turner PC, Mykkänen H, El-Nezami H. Lactobacillus rhamnosus strain GG modulates intestinal absorption, fecal excretion, and toxicity of aflatoxin B(1) in rats. *Appl Environ Microbiol* 2006; **72**: 7398-7400
- 46 **El-Nezami HS**, Polychronaki NN, Ma J, Zhu H, Ling W, Salminen EK, Juvonen RO, Salminen SJ, Poussa T, Mykkänen HM. Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from Southern China. *Am J Clin Nutr* 2006; **83**: 1199-1203
- 47 **Liong MT**. Safety of probiotics: translocation and infection. *Nutr Rev* 2008; **66**: 192-202
- 48 **Salminen MK**, Tynkkynen S, Rautelin H, Saxelin M, Vaara M, Ruutu P, Sarna S, Valtonen V, Järvinen A. Lactobacillus bacteremia during a rapid increase in probiotic use of



- Lactobacillus rhamnosus GG in Finland. *Clin Infect Dis* 2002; **35**: 1155-1160
- 49 **Dugoua JJ**, Machado M, Zhu X, Chen X, Koren G, Einarson TR. Probiotic safety in pregnancy: a systematic review and meta-analysis of randomized controlled trials of Lactobacillus, Bifidobacterium, and Saccharomyces spp. *J Obstet Gynaecol Can* 2009; **31**: 542-552
- 50 **Cannon JP**, Lee TA, Bolanos JT, Danziger LH. Pathogenic relevance of Lactobacillus: a retrospective review of over 200 cases. *Eur J Clin Microbiol Infect Dis* 2005; **24**: 31-40
- 51 **Salminen MK**, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, Järvinen A. Lactobacillus bacteremia, clinical significance, and patient outcome, with special focus on probiotic L. rhamnosus GG. *Clin Infect Dis* 2004; **38**: 62-69
- 52 **Martin FP**, Sprenger N, Yap IK, Wang Y, Bibiloni R, Rochat F, Rezzi S, Cherbut C, Kochhar S, Lindon JC, Holmes E, Nicholson JK. Panorganismal gut microbiome-host metabolic crosstalk. *J Proteome Res* 2009; **8**: 2090-2105
- 53 **Martin FP**, Wang Y, Sprenger N, Yap IK, Lundstedt T, Lek P, Rezzi S, Ramadan Z, van Bladeren P, Fay LB, Kochhar S, Lindon JC, Holmes E, Nicholson JK. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol* 2008; **4**: 157
- 54 **Dumas ME**, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci USA* 2006; **103**: 12511-12516
- 55 **Li M**, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, Zhang Y, Shen J, Pang X, Zhang M, Wei H, Chen Y, Lu H, Zuo J, Su M, Qiu Y, Jia W, Xiao C, Smith LM, Yang S, Holmes E, Tang H, Zhao G, Nicholson JK, Li L, Zhao L. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci USA* 2008; **105**: 2117-2122

S- Editor Tian L L- Editor Stewart GJ E- Editor Zheng XM