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Probiotics: where are they going next? New and emerging areas of research

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Introduction

Probiotics have been defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (WHO/FAO Joint Working Group 2002). Over the years, it has become clear that the gut microbiota is closely linked with health. Probiotics have many proven health benefits, and their mechanisms of action are now becoming better understood, even at a molecular level.

Research surrounding probiotics has historically focused on digestive health. However, over recent years, scientists have been investigating the potential immune benefits of probiotics, as well as other health benefits beyond the recognised area of the gut.

This paper reviews some of these new areas such as the possible role of probiotics for sportspeople, for obesity-related disorders, or for people with alcoholic liver disease (ALD) or autism, where, although the evidence is not conclusive, positive results are emerging.

Exploring the benefits of probiotics

Probiotics support the work of other beneficial bacteria in the gut, and extensive research has been carried out looking at their effect on the immune system. Few people realise that 85% of the body's lymph nodes can be found in the gut (McDonald & Bateman 2007), making it the body's largest immune organ. With a surface area of more than 200 m^2 (ten times that of the skin), because of the numerous villi and microvilli, the gut must be able to mount a defence against the pathogens that it encounters (Holzapfel *et al*. 1998). Previous trials with probiotics tend to show immune benefit in individuals who already have a weakened immune system. For example, in a double-blind, placebo-controlled trial by Morimoto *et al*. (2005), an intervention of *Lactobacillus casei* Shirota increased the number of natural killer (NK) cells in smokers. In another trial investigating the effect of probiotic *Lactobacillus reuteri* Protectis ATCC55730 on short-term sick leave due to respiratory and gastrointestinal (GI) infections, it was the shift workers (who have been shown to be at a significantly higher risk of attracting short-term illnesses) who showed the biggest reduction in illnesses after a course of probiotics (Mohren *et al*. 2002; Tubelius *et al*. 2005).

Sportspeople

Sportspeople may also have impaired immune systems, and this has led to researchers exploring the potential benefits of probiotics for this group. Elite athletes are often more susceptible to coughs and colds, which can be detrimental to training and competing. This could be due to several reasons, such as poor nutrition, extreme conditions, lack of sleep and psychological stresses. All of these can impair the immune system, leaving the athlete prone to picking up infections. This has been termed by some researchers as 'exercise-induced immune depression' and has stimulated research in this area. Stress has been shown to affect levels of total salivary immunoglobulin A (IgA), and correlations have been observed between a reduction in IgA during training and an increase in the number of upper respiratory tract infections in elite athletes (Gleeson *et al*. 1999, 2000). Other studies have shown that after intense exercise, secretory IgA (which is a major effector of host resistance to upper respiratory tract infections) decreases in concentration and flow rate (McDowell *et al*. 1992).

Although there have been limited trials with probiotics in athletes, most of the results have been positive. One of the biggest trials conducted was with 141

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marathon runners. This double-blind, placebo-controlled trial used the probiotic *Lactobacillus rhamnosus* GG during 3 months of summer training. Although there was no difference in incidence of respiratory tract infections or GI episodes, there was a trend for shorter GI problems during training and significantly shorter periods of GI disturbances after the marathon (Kekkonen *et al*. 2007).

Another trial, this time with *Lactobacillus casei* DN-114 001, was conducted by Tiollier *et al*. (2007) with 47 army cadets during a 3-week training course and a 5-day combat course. The results showed a significant increase in dehydroepiandrostane sulphate (an immunostimulatory hormone) in the probiotic group after the combat course ($P < 0.05$). There was also a significant decrease in salivary IgA concentration in the placebo group after the course ($P < 0.01$), but in the probiotic group, there was no significant change.

Other studies in sportspeople have indicated an improvement in the level of NK cells when taking a probiotic (Pujol *et al*. 2000). An increase in IFN-γ (a cytokine involved in defence against viral infections) was also observed when taking a probiotic (Clancy *et al*. 2006; Cox *et al*. 2008). A further study using the probiotic *Lactobacillus casei* Shirota in cyclists found that the probiotic group had increased circulating CD4+ cells and an improved CD4+/CD8+ ratio at rest and post-exercise. CD4+ T cells are helper T lymphocytes involved in the activation of the acquired immune response to pathogens, and CD8+ T cells are suppressor and cytotoxic T lymphocytes. The CD4+/CD8+ ratio is a common marker of immune status (Gleeson 2008).

Obesity

Obesity is a complex and multifactorial disease, which negatively impacts on health. Scientists have become interested in the relationship between the intestinal microbiota and obesity. Although the root cause of obesity is excess calorie intake compared with expenditure, differences in gut microbial ecology among humans may be an important factor affecting energy homeostasis. It has been suggested that individuals predisposed to obesity may have a gut microbiota that promotes more efficient extraction and/or storage of energy from a given diet, compared to lean individuals (Ley *et al*. 2005). Research has shown that the gut bacterial flora of obese mice and humans is different to that of their lean counterparts (DiBaise *et al*. 2008), while Kalliomäki *et al*. (2008) showed that overweight children have lower numbers of faecal bifidobacteria and higher numbers of *Staphylococcus aureus* than those of a healthy weight. More research is needed to determine the cause and effect relationship between the gut flora and obesity, that is, could the gut flora be one of the causes of obesity or is the distorted gut flora a result of obesity, the cause of which could be a poor diet.

Obesity and metabolic disorders such as type 2 diabetes and insulin resistance are linked to inflammation (Hotamisligil *et al*. 1993; Kahn & Flier 2000; Wellen & Hotamisligil 2005), and recent studies have implicated lipopolysaccharide (LPS) (part of the cell membrane of Gram-negative bacteria, and an endotoxin) as a trigger. A high-fat diet was reported to induce an inflammatory response and metabolic disease in mice, which was associated with higher plasma endotoxin levels (*i.e*. metabolic endotoxaemia) (Cani *et al*. 2007a). The high-fat diet was also linked to reduced numbers of bifidobacteria in the gut and an increase in the proportion of Gram-negative bacteria. Mice on the high-fat diet were then fed a prebiotic (oligofructose), which restored bifidobacterial numbers and reduced the high endotoxin levels. Endotoxaemia negatively correlated with *Bifidobacterium* spp., which positively correlated with improved glucose tolerance, glucose-induced insulin secretion and less endotoxaemia, plasma and adipose tissue proinflammatory cytokines (Cani *et al*. 2007b).

Because probiotics are able to positively alter the balance of bacteria in the gut, researchers have hypothesised that they may have a potential role in weight loss. A control group study by Martin *et al*. (2008) showed that probiotics affect the way in which bile acids are metabolised, which in turn can alter the amount of fat that the body is able to absorb. In the study, mice were fed either *Lactobacillus paracasei* or *Lactobacillus rhamnosus*, while controls were given a saline drink. The mice fed either of the probiotics had a higher gut content of bile acids, compared to the controls, which in turn resulted in lower intestinal absorption of dietary lipids and a reduction of lipoprotein levels in plasma. This area of research is relatively new but suggests that probiotics may have a beneficial effect on host metabolism.

The potential antilipolytic effect of probiotics has also been investigated. It is well known that adipose tissue secretes cytokines known as adipocytokines (Karbowska & Kochan 2006; Rosen & Spiegelman 2006). These include leptin and adiponectin, which are known to act as regulators of energy homeostasis. Generally, the concentration of leptin in serum is positively associated with the mass of adipose tissue and adipocyte size, while the serum adiponectin level is negatively associated with the adipose tissue weight. Leptin functions as part of a feedback mechanism that suppresses

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appetite through its receptor at the hypothalamus. A recent study using a milk product fermented by a probiotic bacteria, *Lactobacillus gasseri* SBT2055, found that rats fed the experimental diet with probiotic had greater numbers of small adipocytes and 32% lower serum leptin levels compared to the control group ($P < 0.05$) fed the same diet but with skimmed milk powder, indicating a possible beneficial effect on the onset of obesity by influencing the size of the cells from visceral adipose tissue (Sato *et al*. 2008).

Liver disease

Alcoholic liver disease (ALD) is another new and emerging area of probiotic research. Statistics show that mortality rates for deaths related to alcohol consumption have been increasing in England and Wales. In 2000, there were over 5500 alcohol-related deaths, and of these, 85% were caused by chronic liver disease and cirrhosis (Office for National Statistics 2004).

Patients with ALD are susceptible to a variety of complications and have a reduced life expectancy. Depending on the severity of cirrhosis, 1-year mortality ranges from 10% to 82% (Mansour *et al*. 1997). The most common factor that precipitates the complications is infection, and once infected, patients have increased in-hospital mortality (Linderoth *et al*. 2006).

Increased susceptibility to infection may be caused by endotoxin leaking from the gut, accumulating in the blood and not being cleared by the liver. Endotoxin is known to be high in the peripheral blood of ALD patients. Research has shown that endotoxaemia contributes to neutrophil dysfunction, infection risk and mortality in patients with alcoholic cirrhosis (Mookerjee *et al*. 2007). Neutrophils are phagocytic white blood cells, which are part of the innate immune system (the body's first line of defence) and therefore play an important role in the body's response to infection.

Trials have shown that probiotics may be beneficial to patients with alcoholic cirrhosis by decreasing hepatic encephalopathy (Liu *et al*. 2004; Dbouk & McGuire 2006), improving liver biochemistry (Lirussi *et al*. 2007) and decreasing the rate of infections after liver transplantation and other surgery (Rayes *et al*. 2002; Sugawara *et al*. 2006).

Increased endotoxaemia in patients with cirrhosis is thought to be as a result of greater gut permeability leading to increased bacterial translocation and altered gut flora with a predominance of Gram-negative bacteria (Wiest & Garcia-Tsao 2005). As probiotics are known to positively alter gut flora and decrease Gram-negative organisms, researchers have hypothesised that probiotic treatment in patients with ALD could restore neutrophil function and play a role in reducing infection rates.

This hypothesis was tested in a small open-label study in which 12 patients with alcoholic cirrhosis received a probiotic fermented milk drink containing *Lactobacillus casei* Shirota three times a day for four weeks (Stadlbauer *et al*. 2008). The baseline neutrophil phagocytic capacity was significantly lower in patients compared with controls. This normalised in the treatment group after drinking the probiotic drink. In addition, a reduction of endotoxin in the blood was indicated by a significant reduction in toll-like-receptor (TLR4) surface expression compared to baseline. TLR4 is a receptor on the surfaces of immune cells, which recognises LPS. The study concluded that treatment with a *Lactobacillus casei* fermented milk drink for four weeks was safe and normalised the neutrophil phagocytic capacity and the predominantly anti-inflammatory cytokine response to LPS. The data suggested an important mechanistic role for TLR4 in the mediation of neutrophil functional defect, and established a proof-of-concept that probiotics restore innate immune function in cirrhosis.

Autism

Autism is a condition that is part of the autism spectrum disorder (ASD) and is a lifelong condition that develops in early childhood, with a prevalence rate of 90 in 10,000 (Green *et al*. 2005). Individuals with autism often have a long history of broad-spectrum antibiotic use; many autistic children also suffer from GI problems such as bloating, abdominal pain, diarrhoea and constipation (Horvath & Perman 2002).

It has been suggested that multiple courses of antibiotics could disrupt the gut flora, leading to an increase in the number of unfavourable toxin-producing bacteria. Finegold *et al*. (2002) analysed the faecal flora of children with regressive autism and found that their levels of clostridia were higher when compared with control children. In addition, Parracho *et al*. (2005) observed significantly higher numbers of *Clostridium histolyticum* in a group of children with ASD compared with two control groups of healthy siblings and an unrelated group ($P < 0.05$ and $P < 0.01$, respectively). The *Clostridium* genus produces strong neurotoxins, which could potentially cause damage to the brain, perhaps resulting in repetitive behaviour, communication problems and other traits typical of autism. An increase of neurotoxin in the bloodstream could produce systemic effects (Bolte 1998). Consequently, this has caused speculation that there could be a link between the gut

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flora of individuals with ASD and behaviour. The research by Parracho *et al.* (2005) showed that high levels of clostridia were significantly associated with GI problems in the ASD group, and many parents of autistic children in the study reported that their children's behavioural problems coincided with bouts of GI disturbances. This could be because children living with autism may be unable to communicate effectively their digestive discomfort, which leads to behavioural problems such as frustration.

There are very few data on the potential benefits of probiotics for autism. However, in 2006, the results of an unpublished, blind, placebo-controlled study in which 40 autistic children received either a probiotic or a placebo were widely reported in the media. The researchers compared the faeces of autistic children with the faeces of non-autistic children and found the former had raised levels of clostridia in their faeces. The results showed that the probiotic group had decreased levels of clostridia in their stools, and the parents also described a positive effect on their child's mood and general behaviour (*Guardian* 2006). While this is not hard scientific evidence, it adds to the studies that suggest a link between higher levels of clostridia and GI problems in autistic children (Dixon 2006), although it is not certain whether this is a cause or an effect. This is an area worthy of more investigation, to establish whether manipulation of the gut flora with a probiotic could reduce the number of clostridia, and in turn reduce the incidence of GI problems and perhaps even behavioural problems associated with autism.

Conclusion

It is clear from the preliminary evidence reviewed here that probiotics potentially have a role to play in a number of areas of health. For sportspeople, future investigations could explore the idea that if probiotics can produce an improvement in immune parameters, does this result in less sickness, a healthier athlete, and most importantly better results when competing? For obesity, while there is no single solution to the epidemic, probiotics and the gut microbiota might have a role for some obesity-related disorders, although the cause-and-effect relationship needs to be investigated further. Probiotic reduction of levels of blood endotoxin may be of benefit in other conditions where gut permeability is increased and the gut flora is compromised. The positive results reviewed in this paper, mainly from mechanistic and pilot studies, highlight that there is sufficient evidence to warrant further studies of a larger scale.

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