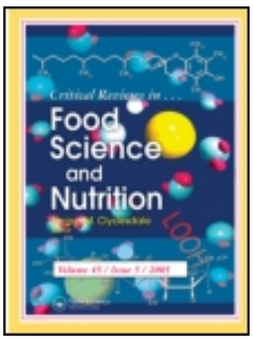

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Impact of Probiotics on Risk Factors for Cardiovascular Diseases. A Review

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Probiotic microorganisms have historically been used to rebalance disturbed intestinal microbiota and to diminish gastrointestinal disorders, such as diarrhea or inflammatory bowel diseases (e.g., Crohn's disease and ulcerative colitis). Recent studies explore the potential for expanded uses of probiotics on medical disorders that increase the risk of developing cardiovascular diseases and diabetes, such as obesity, hypercholesterolemia, arterial hypertension, and metabolic disturbances such as hyperhomocysteinemia and oxidative stress. This review aims at summarizing the proposed molecular and cellular mechanisms involved in probiotic–host interactions and to identify the nature of the resulting beneficial effects. Specific probiotic strains can act by modulating immune response, by producing particular molecules or releasing biopeptides, and by modulating nervous system activity. To date, the majority of studies have been conducted in animal models. New investigations on the related mechanisms in humans need to be carried out to better enable targeted and effective use of the broad variety of probiotic strains.

Keywords Probiotic bacteria, cardiovascular disease, obesity, lipid metabolism, cholesterol, oxidative stress, review

INTRODUCTION

The efficient use of modern sterilization and sanitation technologies in food preparation and conservation has greatly reduced the incidence of food-borne pathogenic microorganisms, specifically infectious diarrhea, listeriosis, and botulism. However, counterbalancing modifications in immune system development could potentially mitigate these gains. According to the "hygiene theory" (Strachan, 1989; Liu and Leung, 2006), a modern and sanitized lifestyle could be an important factor contributing to the increase in allergic diseases. In fact, in developed countries, the gut-associated lymphoid tissue (GALT) is less exposed to, and thus less stimulated, by environmental or pathogenic microorganisms (Vinod et al., 2009). It might, therefore be beneficial to reintroduce some selected species of bacteria, as probiotics, back into the human food diet in order to promote and reinforce our natural defenses (Dave and Shah, 1997; Gardiner et al., 1998; Erkkil et al., 2001; Betoret et al., 2003; Ouwehand et al., 2004).

This concept of creating beneficial health effects through the ingestion of living bacteria has been derived primarily from original studies made by Metchnikoff in 1907 (Metchnikoff, 1907). He suggested that the good health and longevity of certain ethnic groups were due to their frequent ingestion of fermented dairy products. A joint working group, combining the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO/WHO, 2002), has defined these probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." These microorganisms,

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usually bacteria, are especially involved in improving general gut health and positively influencing the development and activity of the immune system (Fuller, 1991; Kaur et al., 2002). Microorganisms commonly used as probiotics are bifidobacteria (Agrawal et al., 2008), lactobacilli (Yea Ping et al., 2008), lactococci (Nishitani et al., 2009), streptococci (Power et al., 2008), enterococci (Lund et al., 2002), *Escherichia coli* (Kruis et al., 2004), and yeasts, particularly *Saccharomyces boulardii* (Guslandi et al., 2000).

The beneficial effects of probiotics have been documented, but such documentation often remains disparate and strain-specific. While some species have been shown to confer beneficial effects, e.g., the treatment of acute diarrhea associated with rotavirus (Isolauri et al., 1991), ulcerative colitis (Ishikawa et al., 2003; Kruis et al., 2004), *Clostridium difficile*-associated diarrhea (McFarland et al., 1994), and *Helicobacter pylori* infection (Nista et al., 2004; Wang et al., 2004); prevention of antibiotic-associated diarrhea in children (Szajewska et al., 2006); and improvement in lactose digestion (de Vrese et al., 2001), others potential effects are still under investigation: liver disease, allergy, AIDS (Baker and Day, 2008; Dominguez-Bello and Blaser, 2008; Sleator and Hill, 2008).

If there is good evidence that probiotics are effective in preventing gastrointestinal disorders, the efficacy of probiotics have not been proven in the treatment of diseases and a number of questions remain unanswered. First, what is the mechanism of bacterial entry and survival in the gastrointestinal tract? In order to survive and remain active, probiotic strains destined for human consumption must be able to withstand both industrial processing procedures (Mille et al., 2004; Meng et al., 2008) and prevailing conditions in the digestive tract (stomach acidity, bile salt, enzymes, and variable amounts of available oxygen). Second, what are the physiological mechanisms in the gut in response to the presence of living bacteria? In practice, a probiotic dose [10^9 (colony-forming units) CFU daily intake] must be able to adhere to the epithelium and to interact with the resident microbiota throughout the digestive human tract. The human gut microbiota is an extremely diverse environment, containing 10^{14} bacteria and ~600 bacterial species divided into three main phyla: Firmicutes (*Clostridium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*), Bacteroidetes (*Bacteroides*), and Actinobacteria (*Bifidobacterium*) (Eckburg et al., 2005).

The specifics of probiotic mechanisms of action remain largely unknown. The emergence of appropriate genetic tools, however, allows us to better study these effects and to improve on the selection of new probiotic strains. A number of previous studies addressing these concerns have proceeded along three major themes: (1) production of specific molecules (vitamins, enzymes) (Sengl et al., 2006), (2) interaction with indigenous microbiota (competition with pathogens, production of bacteriocins) (Collado et al., 2007; Corr et al., 2007), and (3) interaction with host cells, (Hooper et al., 2002) particularly those of the immune system (Corthesy et al., 2007; Round and Mazmanian, 2009). This last area involves particularly the induction of cytokines (pro- or anti-inflammatory) at the origin of specific immune responses.

Recent studies, performed in vitro or in rodents, have demonstrated the potential positive effects of probiotics on lifestyle-derived disorders, characterized by nutritional imbalances. Stress, overworking, and smoking, in conjunction with a high-calorie and low-nutrition diet, may disrupt intestinal homeostasis and weaken the body's natural defenses (Figure 1). In the long term, this can lead to metabolic syndrome, which is a combination of heart attack risk factors. The National Cholesterol Education Program's Adult Treatment Panel III (ATP III) report identified six components of the metabolic

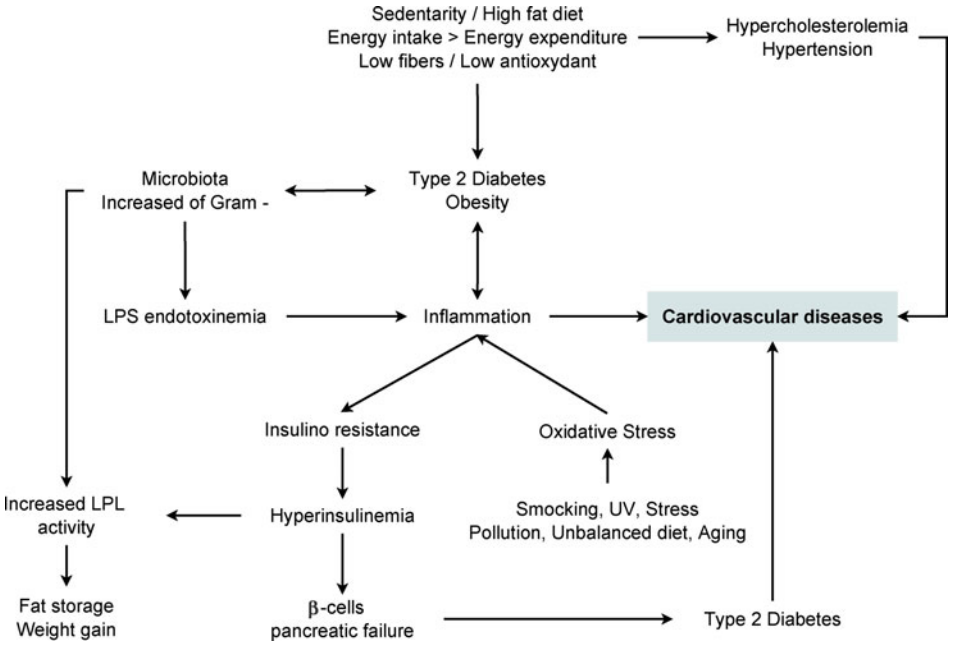

Figure 1 Nutritional and environmental factors leading to cardiovascular diseases. (Color figure available online.)

syndrome that relate to cardiovascular diseases (Grundy et al., 2004; Eckel et al., 2005): abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance, proinflammatory state, and prothrombotic state. Cardiovascular diseases are a class of medical disorders that include aneurysm, angina, atherosclerosis, cerebrovascular accident, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial infarction, and peripheral vascular disease (Rosamond et al., 2008). According to the WHO, these diseases are the leading cause of worldwide morbidity and mortality. Several key dietary factors can lower the risk of cardiovascular diseases, including lowering of low-density lipoproteins (LDL) cholesterol by reducing saturated fat intake, lowering of triglyceride concentrations by reducing sugar and processed foods consumption, reducing homocysteine concentrations by supplementation with vitamins B6 and B12 and folic acid, or increasing antioxidant activity by higher consumption of fruits and vegetables (Hill et al., 2009). In this context, it is necessary to determine whether bacterial action on the intestinal epithelium might also play a role in the risk of cardiovascular diseases. The purpose of this review is to examine the impact of food consumption of probiotic strains on four medical disorders (obesity, diabetes, hypercholesterolemia, and arterial hypertension) implicated in elevating the risk of developing cardiovascular disease. Two metabolic disturbances (hyperhomocysteinemia and oxidative stress) are also examined because of their close relationship with metabolic syndrome. This will be done throughout by presenting a definition of each disorder/disturbance, a review of in-vitro and animal studies, and, when they exists, a review of applicable clinical trials in humans.

PROBIOTIC EFFECTS ON MEDICAL DISORDERS

Impact on Obesity

Obesity is a medical condition defined as an abnormal or excessive fat accumulation that might impair health (WHO, 2000) and which can be associated with an inflammation of the adipose tissue (Wellen and Hotamisligil, 2003). Environmental, behavioral, and genetic factors have been shown to contribute to the development and progression of obesity. In general terms, obesity results from a disequilibrium in energy balance (energy intake, expenditure, and storage) (DiBaise et al., 2008) and can lead to secondary metabolic complications such as Type 2 diabetes, cardiac diseases, and cancer. Reduced physical activity or increased caloric intake both contribute significantly to the energy balance in an individual. This regulation can be controlled by the hormonal system (leptin) and/or perhaps by intestinal bacteria (Figure 2).

Link Between the Microbiota and Obesity

Recent evidence suggests that some bacteria normally found as part of the gut microbiota positively affect nutrient uptake and

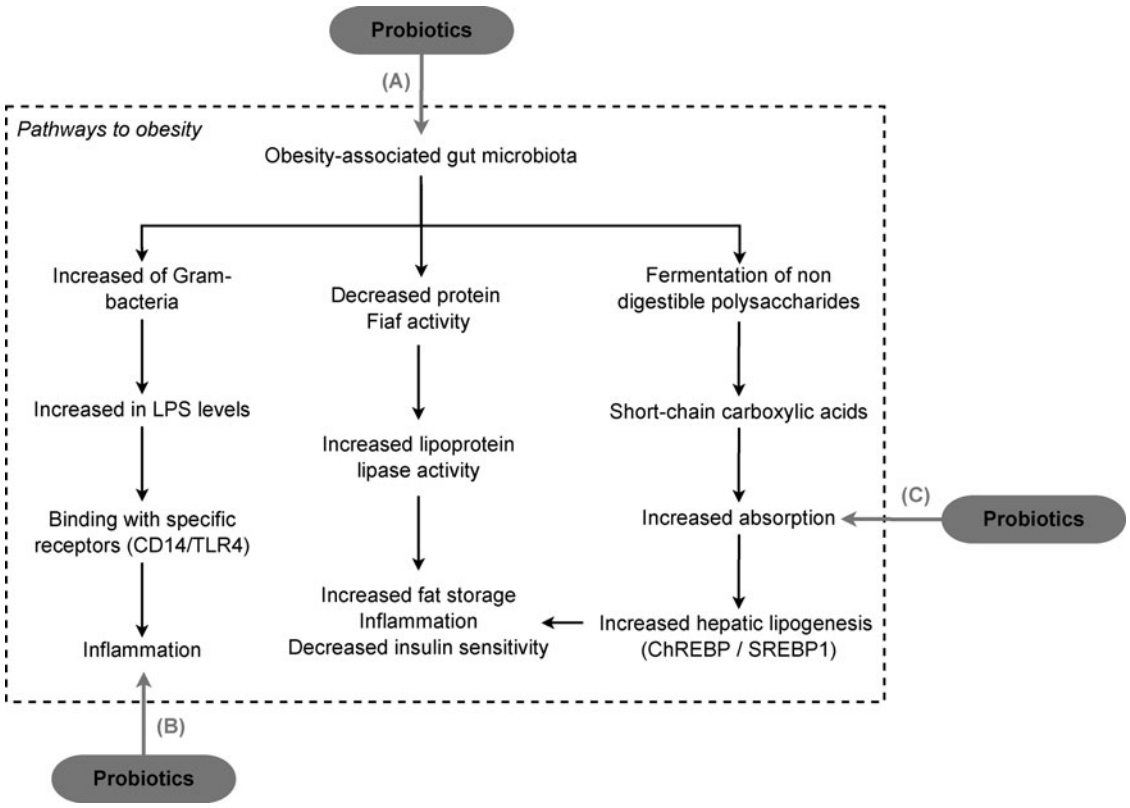

Figure 2 Potential modes of probiotic action on obesity. Probiotics can restore resilient microbiota (A), limit inflammation by their anti-inflammatory properties (B), or reduce fat absorption (C).

energy regulation (Tilg et al., 2009). These findings suggest that the gut microbiota could play an important role in regulating weight and may be partly responsible for the development of obesity in some people (DiBaise et al., 2008). In support of this reasoning, Turnbaugh et al. (2006) demonstrated that the microbiota of obese mice has an increased capacity to absorb energy from a given diet. Vijay-Kumar et al. (2010) further postulated that alterations in the gut microbiota resulting from a loss of toll-like receptor 5 (TLR5) could promote the development of metabolic syndrome in mice. Moreover, the increased caloric needs of germ-free animals have been used by researchers to demonstrate mechanisms involved in energy metabolism and maintenance of body weight. Indeed, the presence of intestinal microbiota contributes to an increase in the absorption of carbohydrates and lipids, by fermenting substrates passing through the colon but otherwise unavailable to the host (Bäckhed et al., 2005), and also helps to regulate fat storage (Bäckhed et al., 2004). Shortly after birth, germ-free mice have reduced adipose tissue volume compared with conventional mice. The subsequent colonization of germ-free mice with intestinal microbiota can lead to a 60% increase of body fat and an increase in insulin resistance in 2 weeks despite a 30% reduction in food intake. Several mechanisms have been proposed to explain this phenomenon: an increase in monosaccharide absorption in the intestine leading to hepatic lipogenesis, an increase in available calories in the form of energy liberated through bacterial fermentation of nondigestible food, or an increase in insulin concentrations, which contributes to anabolism (Bäckhed et al., 2004; Cani and Delzenne, 2009).

The intestinal microbiota might also inhibit the expression of an essential metabolic protein, the fasting-induced adipose factor (FIAF), which specifically inhibits lipoprotein lipase. In this case, the presence of gut microbiota leads to increased lipoprotein lipase activity, which in turn results in the accumulation of fat in the adipose tissue (Bäckhed et al., 2004). It has also been observed that the gut microbiota of obese mice has a higher proportion of bacteria belonging to the Firmicute phylum and a proportionally lower population of Bacteroidetes (Ley et al., 2005). This observation has been confirmed in humans (Ley et al., 2006), and recently extended to obese children (Kalliomaki et al., 2008). These data suggest the existence of a link between the composition of the intestinal microbiota and obesity. More generally, the composition of the microbiota, as measured by the Firmicutes:Bacteroidetes ratio, seems to have an impact on the health of the host. However, it is yet unclear as to whether these compositional differences exist as a cause or the consequence of obesity (Flier and Mekalanos, 2009; Reinhardt et al., 2009).

Immunomodulatory Properties

Clinical obesity, in addition to its obvious outward phenotype, is now recognized as a low-grade inflammatory condition associated with increased macrophage infiltration of the adipose tissue (Wellen and Hotamisligil, 2005; Caropreso et al., 2006; Heilbronn and Campbell, 2008). The compositional alterations in the intestinal microbiota, and particularly the decrease in bifidobacteria, in obese individuals leads to an increase in the lipopolysaccharide plasma concentration. This elevated level of lipopolysaccharides in turn stimulates the synthesis and secretion of proinflammatory cytokines (Cani et al., 2007). The adipose tissue itself also contributes to inflammation through the production of proinflammatory cytokines (IL-6, TNF-α, adiponectin) and other compounds such as leptin, which contribute to insulin resistance (DiBaise et al., 2008). Given the measurable perturbations in the microbiota associated with obesity, the consumption of some probiotics potentially represents a novel tool of risk reduction. Indeed, probiotic use would seem particularly suited to restoring resilient microbiota (Figure 2A) and reducing inflammation (Figure 2B) which, if left untreated, could induce a nonreversible disruption and a potential increased sensitivity to infections. Some strains such as *E. coli* Nissle 1917, *Lactobacillus rhamnosus* GG, and *Faecalibacterium prausnitzii* can act on the immune system with anti-inflammatory effect (Korhonen et al., 2001; Schultz et al., 2004; Sokol et al., 2008). Some bacterial strains have been shown to interact with nuclear receptors (α, β, γ), and especially PPAR-γ (Kelly et al., 2004), which plays an important role in lipid storage stimulation (Wellen and Hotamisligil, 2005). However as this point, a direct link between the gut microbiota and the endocrine system remains tentative (Ali et al., 2004).

Enzymatic Activity and Lipid Metabolism

The influence of probiotics on obesity can also be tied to bile salts metabolism and to the reduction in fat absorption by the host (Begley et al., 2005) (Figure 2C). A study in mice has demonstrated the influence on lipid metabolism of the strains *L. paracasei* NCC2461 and *L. rhamnosus* NCC4007 (10^8 CFU/day). A decrease in the plasma concentrations of very-low-density lipoproteins (VLDL) and LDL was observed under this regime, along with a complementary increase in triglyceride concentrations. These observed changes were likely due to the induced modification in the enterohepatic recirculation of bile acids, which have been shown to lower cholesterol and systemic concentration of blood lipids (Martin et al., 2008). Other studies have also reported on the ability of some probiotics (*L. acidophilus* CCRC 14079, *L. acidophilus* LA-5, *L. casei* NCDC 19, and *Bifidobacterium animalis* BB-12) to produce conjugated dienes of linoleic acid (CLA) through linoleic acid isomerase (Lin et al., 2003; Akalin et al., 2007; Yadav et al., 2007b). CLA compounds have been reported to possess antiobesity activity (West et al., 1998; Terpstra, 2004; Wang and Jones, 2004; Macouzet et al., 2009). However, the decrease in body fat due to the consumption of exogenous CLA is associated with insulin resistance, hyperinsulinemia, and severe hepatic steatosis (Besnard et al., 2005). Nevertheless, a recent study taking into account the potential risks of CLA showed that ingestion of *L. rhamnosus* PL60 over 8 weeks (1×10^7 CFU/day) reduced the weight of obese mice without a reduction in food intake.

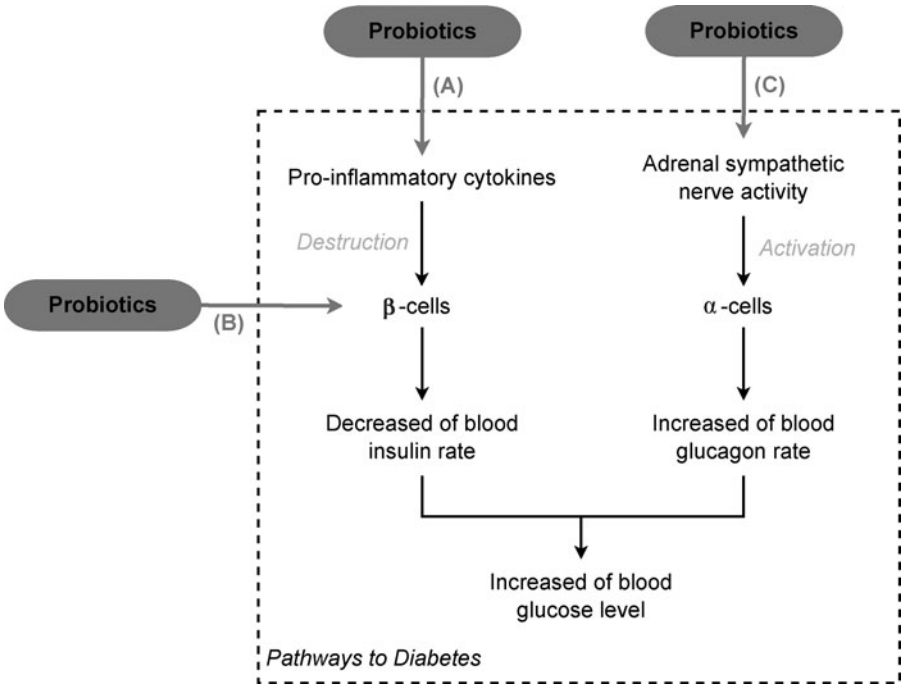

Figure 3 Potential modes of probiotic action on Type 1 diabetes. Probiotics can prevent destruction of β-cells by their anti-inflammatory properties (A), increase insulin production (B), or suppress adrenal sympathetic nerve activity (C).

Most importantly, this weight and adipose tissue reduction was achieved without the development of hepatic steatosis (Lee et al., 2006). Along the same lines, the use of microorganisms with specific enzymatic activity known for their antiobesity effects (e.g., delta-6-desaturase, an enzyme required for the synthesis of highly unsaturated fatty acids) could have potential benefits and should also be studied.

Concluding Remarks

Studies on germ-free animals has allowed for the elucidation of the relationship between the intestinal microbiota and the host metabolism, especially in the case of obesity. The importance of the microbiota composition in obesity, and particularly the Firmicutes:Bacteroidetes ratio, has been shown in model laboratory organisms and confirmed in humans. The effect of probiotics on obesity in mice appears promising as test animals experience a positive change in the composition of their intestinal microbiota accompanied by alterations in metabolism. Up to now, extrapolation of these encouraging results has not been possible due to the lack of human data and due to a controversial debate linking probiotics and weight gain (Delzenne and Reid, 2009; Raoult, 2009).

Impact on Diabetes

Diabetes is a chronic disease that occurs when the pancreas does not produce any insulin (Type 1 diabetes), or when the body cannot effectively use the insulin produced, and/or when the pancreas does not produce enough insulin (Type 2 diabetes) (WHO, 1999). Type 1 diabetes is an autoimmune disease resulting in a total destruction of the insulin-secreting β-cells of the islets of Langerhans in the pancreas (Yoon and Jun, 2005). The destruction of β-cells leads to a lack of insulin in blood, leading to an increase in glycemia and the necessity of daily insulin treatments. Since $\sim$90% of Type 1 diabetes cases are autoimmune in nature, caused by an overactive immune response, the use of immunomodulatory probiotic strains has been proposed as a method of treatment. To this end, Wen et al. (2008) have shown that the interaction of the microbiota with the immune system is an important factor modifying predisposition to Type 1 diabetes. Figure 3 shows the pathways leading to Type 1 diabetes and the three ways in which probiotics can act on β-cells, immune cells, and the nervous system.

In-Vitro and Animal Studies—Type 1 Diabetes

The oral administration of *L. casei* during 8 weeks (daily uptake unknown) in mice significantly reduced blood glucose concentrations after 12 weeks and also inhibited the production of T lymphocytes (CD4+) and proinflammatory cytokines (IFN-γ and IL-2), molecules implicated in Type 1 diabetes progression (Matsuzaki et al., 1997c) (Figure 3A). Furthermore, oral administration of *L. casei* to mice treated with alloxan (a molecule that specifically targets β-cells of Langerhans islets and thus mimics Type 1 diabetes) can inhibit the destruction of the β-cells (Matsuzaki et al., 1997a, 1997b). A more recent study (Tabuchi et al., 2003) has shown that hyperglycemia may be significantly attenuated by the administration

of *L. rhamnosus* GG (daily uptake unknown) over a 10-week period, where an effect on insulin production and glucose tolerance was observed (Figure 3B). Yamano et al. (2006) have carried out studies on rats supplied water supplemented with a probiotic strain (*L. johnsonii* La1: $\sim 1.9 \times 10^9$ CFU/day) and have demonstrated a decrease in glucose and glucagon amounts, along with an increase in insulin concentrations in the blood. After having administered probiotics by intraduodenal injection in rats (2×10^{10} CFU/day), these authors have revealed a suppression of adrenal sympathetic nerve activity, which could improve glucose tolerance by reducing glucagon secretion (Figure 3C). A further investigation was also undertaken into the application of the probiotic strain *L. acidophilus* ($\sim 1.89 \times 10^9$ CFU/day) with the goal of restoring nitric oxide concentrations in rats, which is an important regulator of many physiological processes. Importantly, nitric oxide bioactivity is known to be low in Type 1 diabetes (Harisa et al., 2009).

In-Vitro and Animal Studies—Type 2 Diabetes

This noninsulin dependent diabetes is a metabolic disorder affecting glucose regulation and eventually causing diabetes mellitus, characterized by insulin resistance and reactive hyperinsulinemia. Rats placed on a high-fructose diet for 8 weeks suffered from glucose intolerance and an increase in glucose, cholesterol [high-density lipoproteins (HDL) and LDL], and triglycerides amounts in the bloodstream (Yadav et al., 2007a). The effect of a fermented milk product containing probiotic strains (*L. acidophilus* and *L. casei*: unknown quantities) demonstrated that a number of diabetes-associated parameters (i.e., glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress) could be improved by ingestion of the probiotics.

Concluding Remarks

Initial studies into the action of probiotic strains on Type 1 or Type 2 diabetes appear promising. Probiotics from a number of reports have been shown to influence Type 1 diabetes at three levels: (1) by regulating immune system activity and thus reducing destruction of pancreatic cells, (2) by simulating insulin production, thereby aiding the reabsorbtion of circulating glucose, and (3) by decreasing glucagon production via modulation of adrenal sympathetic nerve activity. Understanding the relationship between microorganisms and neural modulation is vital to finding new probiotic applications, yet very few studies exist that address this issue (Girard et al., 2009). Current research concentrates primarily on the empirical testing of probiotics strains and in understanding how they act. It would therefore be extremely attractive to focus on specific molecules implicated in diabetes, screening potential probiotic strains for their ability to influence these compounds. A similar screening approach might also be applied toward obesity using genetically diabetic mice (db/db) as a model.

Impact on Hypertension

Nearly a quarter of the population in developed countries suffers from hypertension, another of the risk factors for cardiovascular disease. Even though there are no direct causes of hypertension, there are many risk factors such as sedentary lifestyle, obesity (more than 85% of cases occur in those with a body mass index greater than 25), sodium sensitivity, alcohol intake, and vitamin D deficiency (Sacks et al., 2001; Wang et al., 2008). As a result of prevailing modern trends, the percentage of individuals affected by this condition continues to increase. Since an overactive renin–angiotensin system (RAS) leads to vasoconstriction and retention of sodium and water, a way to reduce hypertension is to use ACE (angiotensin-converting enzyme) inhibitors (Burnier and Zanchi, 2006) (Figure 4). ACE is responsible for increasing blood pressure and blood volume by converting angiotensin I to angiotensin II, a potent vasoconstrictor, and by degrading bradykinin (Gainer et al., 1998), a potent vasodilator, and other vasoactive peptides.

In-Vitro and Animal Studies

Very few studies are currently available on the use of probiotic microorganisms in reducing hypertension and its concordant risk of developing cardiovascular disease. However, beneficial effects of probiotic strains on blood pressure have been publicized in some reports. Kawase et al. (2000) have observed a systolic pressure reduction of 6 mmHg in rats after 8 weeks of *L. casei* TMC 0409 ($\sim 2.4 \times 10^{11}$ CFU/day) and *Streptococcus thermophilus* TMC 1543 ($\sim 10^{10}$ CFU/day) ingestion. The atherogenic index of plasma and triglyceride concentrations was also significantly lowered after 4 weeks. Tanida et al. (2005) have suggested that the probiotic

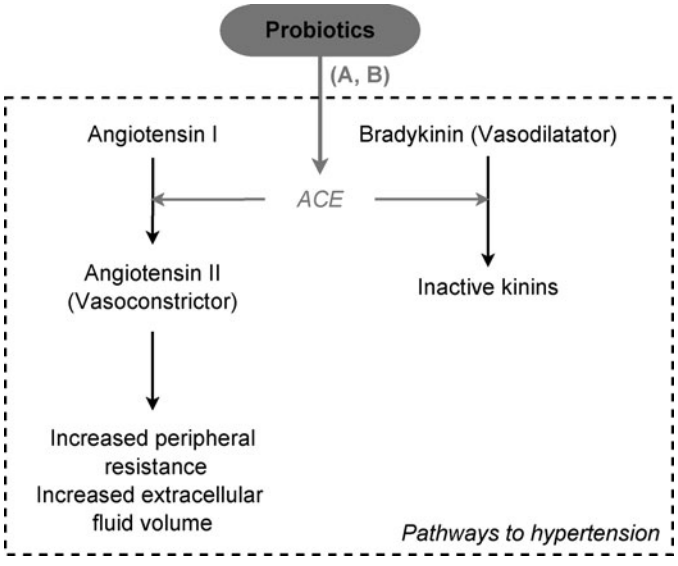

Figure 4 Potential modes of probiotic action on hypertension. Probiotics can release bioactive peptides with antihypertensive properties (A) or inhibit ACE by enzymatic activities (B).

strain *L. johnsonii* La1 (intraduodenal injection: $1 \times 10^{8-9}$ CFU/day), or its metabolites, might lower hypertension by changing autonomic neurotransmission via the central histaminergic nerves and the suprachiasmatic nucleus in rats.

Studies in Healthy Volunteers and Clinical Trials

Probiotic strain *L. plantarum* 299v (2×10^{10} CFU/day), when administered to 36 smokers over 6 weeks (controlled, randomized, double-blind trial), was shown to lower systolic blood pressure (13 mmHg) (Naruszewicz et al., 2002). *L. plantarum* 299v also induced a significant increase in HDL (+10%) and a decrease in leptin concentration (−37%), LDL (−12%), fibrinogen (−21%), IL-6 (−41%), and F_2-isoprostanes (−31%), which are biochemical markers of lipid peroxidation and oxidative stress. All these modifications could help to reduce the cardiovascular risk. Similarly, Seppo et al. (2003), in a randomized placebo-controlled study with 39 hypertensive patients, showed that consumption of the strain *L. helveticus* LBK-16H during 21 weeks led to a significant reduction of 6.7 mmHg in systolic pressure. These results were confirmed in a randomized, placebo-controlled, double-blind trial study involving 40 subjects with high normal blood pressure and 40 subjects with mild hypertension, each receiving *L. helveticus* CM4 over a 4-week period (Aihara et al., 2005). Despite these positive results, Simons et al. (2006) failed to observe a significant reduction in blood pressure after ingestion of *L. fermentum* for 10 weeks (4×10^9 CFU/day). This single-center, double-blind, placebo-controlled parallel design trial was conducted with 46 volunteers with elevated serum cholesterol. It has been proposed that microorganisms are able, through their proteolytic activity, to release bioactive peptides that exhibit antihypertensive properties (e.g., casokinine from milk casein or lactokinine from α-lactalbumin or β-lactoglobulin) and inhibit ACE activity (Donkor et al., 2007) (Figure 4A). These properties are currently limited to certain strains of lactobacilli with specific cell wall enzymes (proteinases) (Flambard, 2002; Nielsen et al., 2009; Sun et al., 2009).

Concluding Remarks

Despite initial positive results of probiotic strains on blood pressure, care must be taken in the interpretation of results, given that hypertension is closely related with other medical disorders (diabetes, cholesterol, obesity). Currently published studies have been limited to examining the capacity of microorganisms to render antihypertensive biopeptides from milk proteins. A new avenue of research might more effectively focus on identifying bacteria capable of directly producing these molecules (Figure 4B). Moreover, since the gut microbiota is implicated in angiogenesis, itself linked to hypertension (Sane et al., 2004), the use of germ-free animals, as in obesity, could provide new insights into the relationship between gut microbiota and hypertension. As some bacteria are known to produce nitric oxide (Sobko et al., 2006), which is an important vasodilator, this capacity might also be targeted to reduce hypertension. Finally, as artery constriction is under control of noradrenalin, the impact of microorganisms on the nervous system (Ait-Belgnaoui et al., 2006) and consequent neurotransmitter release could also prove to be a fruitful avenue of research.

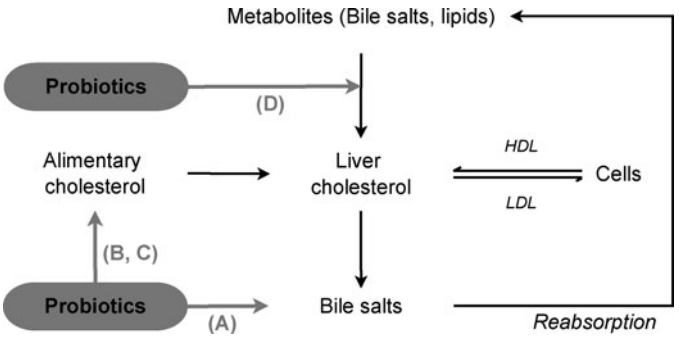

Figure 5 Potential modes of probiotic action on hypercholesterolemia. Probiotics can decrease cholesterol concentration through BSH enzymatic activity (A), incorporate cholesterol into their own membranes (B), convert cholesterol to coprostanol (C), or decrease cholesterol synthesis (D).

Impact on Hypercholesterolemia

For over two centuries, the role of cholesterol in the risk of developing inflammatory diseases such atherosclerosis has been noted (Hlivak et al., 2005). Cholesterol is carried in the bloodstream by lipoproteins such as LDL, VLDL, HDL, and chylomicrons (Figure 5). VLDL and LDL transport cholesterol from the liver to other cells in the body. An increase in the amount of circulating LDL will eventually result in the formation of atherosclerotic plaques (deposits of cholesterol in the arteries), increasing the risk of cardiovascular diseases when associated with oxidative stress (Durrington, 2003). HDL, in the reverse role, collects cholesterol from the arteries and atheroma and returns it to the liver (Gordon et al., 1989).

In-Vitro and Animal Studies

Drouault et al. (2002) attempted to establish a relationship between the structure of the gut microbiota and its ability to influence fat metabolism and assimilate cholesterol. Three mechanisms of action have been proposed: (1) degradation of bile acids through the enzyme bile salt hydrolase (BSH), which deconjugates bile salts and reduces their reabsorption by the body (Gilliland and Walker, 1990) (Figure 5A), (2) membrane incorporation of cholesterol, e.g., by the strain *L. acidophilus* ATCC 43121 (Noh et al., 1997) (Figure 5B), and (3) conversion of cholesterol to coprostanol, which is easily assimilated and excreted in feces (Cardona et al., 2000; Veiga et al., 2005; Gerard et al., 2007) (Figure 5C). Other researchers believe that probiotics could decrease the synthesis of cholesterol in the liver itself (Fukushima and Nakano, 1996; Chiu et al., 2006) (Figure 5D). Most studies to date show a beneficial effect of probiotics on cholesterol concentration. Kawase et al. (2000) observed an

increase in HDL (+28%) and a decrease in triglyceride concentration (−20%) in rats supplemented with *L. casei* TMC 0409 ($\sim 2.4 \times 10^{11}$ CFU/day) and *S. thermophilus* TMC 1543 ($\sim 10^{10}$ CFU/day) for 4 weeks. Similarly, a positive effect on LDL concentration has been observed with the strain *Bifidobacterium longum* BL1 (Xiao et al., 2003), where a significant decrease in the concentration of LDL was observed (−41%) in a group fed with probiotics in comparison with a control group. Wang et al. (2009) evaluated the effects of *L. plantarum* MA2 (10^{11} CFU/day) in mice during 5 weeks and revealed a significantly lowered serum total cholesterol (−21%), LDL (−20%), and triglyceride concentration (−25%), while observing no change in HDL. In addition, total liver cholesterol and triglycerides were also decreased by 21% and 14%, respectively, proving that total cholesterol was reduced and not simply redistributed between the blood and the liver.

Studies in Healthy Volunteers and Clinical Trials

Studies published thus far have documented uncertain effects of probiotics on blood cholesterol concentration (Dacosta, 2001; Pawan and Bhatia, 2007). In 20 tests carried out between 1979 and 2000, 10 studies have shown no significant effects and 10 others reported a reduction in total cholesterol concentration between 5% and 10%. It is worth noting that cholesterolemia can vary by ±5% in a few months without apparent causes, while the measurement accuracy is within 2% to 7% (Dacosta, 2001). Therefore, the aggregate of these results describes a nonsignificant effect of probiotic consumption on blood cholesterol. In contrast, a meta-analysis of data from 425 individuals revealed a significant and beneficial effect of probiotics on reducing cholesterol concentration (a 4% decrease in total cholesterol and a 5% decrease in LDL cholesterol) (Agerholm-Larsen et al., 2000). Furthermore, Xiao et al. (2003) have shown a significant reduction of serum cholesterol among 32 subjects with moderate hypercholesterolemia (serum total cholesterol > 240 mg/dl) in a short-term, single-blind parallel study. Here, consumption over 4 weeks of milk fermented by the strain *Bifidobacterium longum* BL1 (1.1×10^{11} CFU/day) was associated with a reduction in total cholesterol in the whole group and in the moderate hypercholesterolemic group by 2.4% and 5%, respectively. Additionally, ingestion of *Enterococcus faecium* M-74 (2×10^9 CFU/day) in 43 volunteers over 56 weeks of a randomized, double-blind, and placebo-controlled study was associated with a 12% reduction in total cholesterol, a decrease in LDL (−19%), and no change in HDL and triglycerides (Hlivak et al., 2005). Recently, Andrade and Borges (Andrade and Borges, 2009) tested the effects of *L. acidophilus* 145 and *B. longum* BB536 on 34 women and observed a significant reduction in LDL (double-blind, placebo-controlled, crossover study). Similarly, Ataie-Jafari et al. (2009) demonstrated that the consumption of yogurt containing *L. acidophilus* and *B. lactis* for 4 weeks was associated with a significant decrease in total serum cholesterol in comparison with ordinary yogurt. This randomized and crossover trial included 14 healthy moderately hypercholesterolemic subjects.

Concluding Remarks

Although previous studies of the probiotic effect on hypercholesterolemia have often yielded inconclusive or contradictory results, more recent papers suggest a beneficial effect on cholesterol imbalance and appear to confirm in-vitro assays. However, reductions in cholesterol concentrations of less than 10% might not be solely due to the ingestion of probiotics but also be the result of typical cholesterol variation and measurement accuracy. Furthermore, all clinical trials to this point have focused on individuals suffering from hypercholesterolemia. It would be interesting and informative to examine the effects of probiotics on healthy individuals. This would be especially true in cases where an underlying predisposition toward development of hypercholesterolemia exists with a hereditary component.

PROBIOTIC EFFECTS ON METABOLIC DISTURBANCES

Impact on Hyperhomocysteinemia

Many studies have marked a correlation between nutrient deficiencies (mainly due to B-vitamins deficiencies (Strain et al., 2004)) and a high plasma homocysteine concentration, which is considered as a risk factor for cardiovascular diseases (Dinavahi and Falkner, 2004). Hyperhomocysteinemia can have an impact on oxidative stress, hemostasis and endothelial dysfunction, decreased nitric oxide production, and can affect vascular smooth muscles (Hofmann et al., 2001). After having focused on the production by microorganisms of vitamin K (Morishita et al., 1999), a vitamin involved in blood clotting and atherosclerotic plaques (Olson, 1984), researchers recently proposed the use of probiotic strains to reduce hyperhomocysteinemia.

In-Vitro Vitamin Production

Studies have shown that some lactic acid bacteria and species of *Bifidobacterium* are able to produce folates in fermented milk (Crittenden et al., 2003; Pompei et al., 2007). However, some lactobacilli strains used in the production of fermented milk have also been shown to depress the concentration of folic acid in milk (Crittenden et al., 2003). Certain strains of *S. thermophilus* are very good producers of folates, resulting in a four-fold increase in milk (Crittenden et al., 2003). Moreover, a mix of different strains can greatly increase the amount of folates in milk. This increased concentration of folate is due to the metabolic activity of lactic acid bacteria and *Bifidobacterium* species (Rossi et al., 2011). During the fermentation process, some probiotics also produce vitamin B12 (Hugenholtz et al., 2002; Taranto et al., 2003; Molina et al., 2009) and vitamin B2 (Hou et al., 2000). A comparative genome analysis aimed at identifying new

biochemical pathways suggested that the ability of some probiotic strains, especially *L. reuteri*, to produce vitamin B12 is due to an adaptive evolutionary response (Morita et al., 2008). The production of B vitamins by probiotics has also been examined in vivo. Pompei et al. showed that administration of three bifidobacteria (*B. adolescentis* MB 227, *B. adolescentis* MB 239, and *B. pseudocatenulatum* MB 116) at 2×10^8 CFU/day for 2 weeks increased folate concentration in the liver and serum in rats (Pompei et al., 2007).

Studies in Healthy Volunteers and Clinical Trials

A randomized nutritional supplementation trial showed that the consumption by children of both sexes (11 years old) of *L. acidophilus* La1 during 6 weeks in a yogurt matrix (10^{12} CFU/day) was associated with increased folic acid and vitamin B12 and decreased homocysteine in the plasma (Mohammad et al., 2006). The ability of probiotics to produce folic acid in humans has been confirmed in three *Bifidobacterium* strains (*B. adolescentis* DSM 18350, *B. adolescentis* DSM 18352, and *B. pseudocatenulatum* DSM 18353) consumed by 23 healthy volunteers (randomly divided into three groups) at 5.10^9 CFU/day during 30 days in a randomized study (Strozzi and Mogna, 2008). These authors have documented the ability of these probiotic strains to colonize the human intestine and to synthesize, de novo, significant amounts of folic acid. A recent study, however, is more nuanced in its conclusions, as it attributed the increase in B-vitamins content in 33 females to regular consumption of fermented dairy product rather than to a specific intake of probiotics (Fabian et al., 2008). The positive effect of the administration of bifidobacteria has also been studied on the homocysteine levels of hemodialysis patients (Taki et al., 2005). Vitamin-producing probiotics may provide a complementary endogenous source of biomolecules that are not synthesized by mammalian cells. Probiotics are especially useful for the homeostasis of human body, and unlike oral administration of the vitamins, ensures a constant bioavailability (Strozzi and Mogna, 2008).

Concluding Remarks

The role of probiotics in the production and/or availability of vitamins is relatively well documented and represents an ideal area for improving nutrition. However, given the discrepancy between certain studies, the beneficial effect of these bacteria should be verified in a nondairy matrix. Finally, even if in-vitro and in-vivo results are not always correlated, the screening for effective probiotic strains seems relatively simple to implement (enzymatic activity, vitamin production) and should lay the groundwork for clinical studies in humans. It will be most interesting to search for new strains that are able to both effectively colonize the host digestive system and produce vitamins, thus ensuring a constant bioavailability.

Impact on Oxidative Stress

Oxidation reactions are essential for energy production in living cells. However, oxygen can lead to formation of reactive oxygen or nitrogen species (ROS or RNS), which alter lipids, proteins, nucleic acids, and carbohydrates and can provoke cell and tissue damage (Storz and Imlay, 1999). Oxidative stress is caused by an imbalance between ROS production and a biological system's ability to readily detoxify the reactive intermediates or to easily repair the resulting damage (Fridovich, 1998). Even as ROS production is used by the immune system as a means of neutralizing pathogens (Torres et al., 2006), oxidative stress is implicated in many diseases such as gastrointestinal inflammation [i.e., inflammatory bowel disease (IBD)], colic disorders, atherosclerosis, myocardial infarction, stroke, Alzheimer's disease, Parkinson's disease, cirrhosis, dermatitis, diabetes mellitus or Type 2 diabetes, age-related macular degeneration, and retinopathy (Witztum, 1994; Suzuki et al., 2007; Castellani et al., 2008; Mikelsaar and Zilmer, 2009; Banday and Lokhandwala, 2011). Metabolic syndrome, obesity, accelerated aging, and the development of certain tumors have also been linked to oxidative stress (Vincent et al., 2007). Increased ROS initiates several processes involved in atherogenesis (Victor et al., 2009), including expression of adhesion molecules, stimulation of vascular smooth muscle proliferation and migration, apoptosis in the endothelium, oxidation of lipids, activation of matrix metalloproteinases, and altered vasomotor activity (Griendling and FitzGerald, 2003a, 2003b; Harrison et al., 2003; Higashi et al., 2009). A recent publication has demonstrated that the intake of antioxidants may lead to the reduced impact of oxidative stress on the human body (Lin and Yen, 1999).

In-Vitro and Animal Studies

Probiotic strains have been explored for their ability to reduce oxidative stress. In vitro, some probiotic strains can reduce oxidative stress. This is the case for *L. fermentum* ME-3 (DSM 14241), possessing a manganese superoxide dismutase (SOD), which can increase the antioxidative defensives (TAA, the total antioxidative activity; TAS, the total antioxidative status; and glutathione reductase) and scavenge pro-oxidant metals (Kullisaar et al., 2002; Jarvenpaa et al., 2007; Teemu et al., 2008; Mikelsaar and Zilmer, 2009). Some lactobacilli and bifidobacteria also possess in-vitro antioxidant capacities (Lin and Yen, 1999; Lin and Chang, 2000; Wang et al., 2006). These strains are notably able to limit lipid peroxidation and to enhance free radical scavenging. In vivo, the proof of the concept of antioxidative strains protecting mice from inflammation has been shown in several studies using either recombinant lactococci (Han et al., 2006; Han and Fioramonti, 2008) or recombinant lactobacilli (Rochat et al., 2006; Han and Fioramonti, 2008; Watterlot et al., 2010; LeBlanc et al., 2011). These recombinant stains producing SOD or catalase were able to reduce damages induced in TNBS- (trinitrobenzene sulfonic acid) and DSS- (dextran sulfate sodium) induced colitis murine models,

suggesting a potential interest in antioxidative probiotic strains for the prevention and the treatment of IBD. Recently, it has been shown that probiotic strain *S. boulardii* when administrated to rats allowed an upregulation of SOD and glutathione peroxydase (DSouza et al., 2010).

Studies in Healthy Volunteers and Clinical Trials

A very few number of studies have been conducted on the impact of probiotic strains on oxidative stress. In 2003, volunteers ($n = 21$) were selected for a 3-week trial in order to assess the effect of the probiotic *L. fermentum* ME-3 (Kullisaar et al., 2003). Administration of fermented goat milk increased the protection against atherogenicity in the participants. It increased the resisting period against oxidation in lipoprotein fraction, and decreased oxidized LDL level, peroxidized lipoproteins, 8-isoprostanes, and glutathione redox ratio. It also improved total antioxidative activity. A clinical study in 2005 reported on the beneficial effects on oxidative stress of probiotics consumption in healthy individuals (Songisepp et al., 2005). Consumption of *L. fermentum* ME-3 in fermented milk (6.3×10^{11} CFU/day, open, placebo-controlled, $n = 21$) or capsules (1.6×10^{9} CFU/day, double-blind, randomized, placebo-controlled, $n = 24$) over 3 weeks was shown to significantly improve the blood TAA and TAS values: 6% and 9% for fermented milk, and 4% and 2.5% for capsules, respectively. The glutathione redox ratio, an indicator of oxidative stress, also increased during the consumption of the milk containing the probiotic. In addition to the dosage effect, the difference in efficiency between the dry and the wet form of administration can be explained by the synergistic effect of the probiotic with the milk substrate, which contains lactose, minerals, vitamins, and other compounds that can improve the level of intestinal metabolism. The increase in TAA value by ingestion of *L. fermentum* ME-3 has also been shown in vitro (Songisepp et al., 2004). A crossover, randomized clinical trial was carried out with conventional yoghurt and probiotic yoghurt containing *L. casei* on 33 healthy female volunteers (Fabian and Elmadfa, 2007).The results showed a significant reduction in total antioxidant capacity values and increase in malondialdehyde and conjugated dienes values in both tested groups. While SOD activity remained unchanged in both yoghurt groups, catalase and glutathione peroxidase activity significantly decreased in the probiotic-consuming group after 4 weeks.

Concluding Remarks

Even when considering the limited number of studies, probiotic strains seem to be able to exhibit a positive effect on oxidative stress, notably though their anti-inflammatory properties (Sokol et al., 2008). However, the correlation between the decrease in oxidative stress markers and the reduction in medical disorders risk, including atherosclerosis, is more difficult to assess. Moreover, the realization of a single in-vitro test or measurement of a single parameter in vivo seems inappropriate since these results are not always correlated (Bean et al., 2009a, 2009b).

CONCLUSIONS AND FUTURE PROSPECTS

Many studies on probiotics have highlighted the importance of the gut microbiota in gastrointestinal disorders and have more recently begun to focus on associated cardiovascular risks. The review characterizes medical disorders and metabolic disturbances in which obesity might be associated to dysbiosis (Cani and Delzenne, 2011). For this purpose, probiotic strains used as tools to reduce risks factors should be tested further. Probiotics show positive effects on the six disorders/disturbances in in-vitro and in-vivo studies, most notably due to their anti-inflammatory properties or their enzymatic capacities. In each case, the mechanisms whereby the bacteria can affect the host are well documented. However, the lack of double-blind, randomized clinical trials, especially for obesity, diabetes, and oxidative stress, makes definitive conclusions impossible at this time. Furthermore, no studies to date have directly addressed the impact of probiotics on risk factors for cardiovascular diseases, which include aneurysm, angina, atherosclerosis, cerebrovascular accident, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial infarction, and peripheral vascular disease.

The use of predictive and representative screening tools should allow for the selection of new functional classes of probiotic bacteria. In the case of obesity, establishment of an experimental protocol with axenic animals has furthered the understanding of the action of the microbiota and its modulation by probiotic strains. Unfortunately, this rigorous methodology is rarely applied and current trends consist rather in empirically testing probiotics strains against a particular medical disorder where common mechanisms are generally outlined but the precise modes of action are completely unknown. Moreover, many studies have been carried out directly on animal models but the results are not always applicable in humans. An improvement in this methodology would be to use human-microbiota-associated mice (Mater et al., 2005) or to use more representative models (which can naturally develop a given medical disorder) such as domesticated animals. Current research, especially the Human Microbiome Project and the MetaHIT (Metagenomics of the Human Intestinal Tract) (Turnbaugh et al., 2007; Qin et al., 2010), should provide more information on the transfer of in-vitro and animal model data to humans and should permit the development of new predictive models.

In this review, we attempt to highlight the links made between medical disorders and their mechanism of action. This understanding is an essential step toward development of better screens for strains harboring a desired specific activity. In the future, recombinant probiotics (Sybesma et al., 2004; Bermudez-Humaran et al., 2007; Rochat et al., 2007) and the determination of the composition of microbiota of patients suffering from specific medical disorders (Sokol et al., 2008) represent two major

avenues of research. The use of probiotics represents another alternative in order to modify the gut microbiota (Meyer and Stasse-Wolthuis, 2009) to manage food intake for overweight and obese people (DiBaise et al., 2008), to reduce Type 1 diabetes (Cani et al., 2007) and hypercholesterolemia (Liong and Shah, 2005).

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